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SPAWNING AND LARVAL DEVELOPMENT OF A TROPICAL ABALONE *HALIOTIS ASININA* (LINNE)

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ABSTRACT

Desiccation, thermal shock, ultraviolet-irradiated seawater, and hydrogen peroxide, singly or in combination, failed to induce the tropical abalone, Haliotis asinina to spawn viable number of eggs or sperm. However, natural spontaneous spawnings occurred frequently and fertilized eggs with an average size of 180 µm were obtained. Trochophore larvae hatched 5.2-5.5 h after fertilization (27.7-30.2°C). Veliger larvae were observed 8 h after fertilization and achieved creeping ability 30 h after fertilization. Competent larvae settled within 2-3 d on prepared diatom plates and juveniles forming the first respiratory pore were observed after 36 d.

INTRODUCTION

The tropical abalone *Haliotis asinina* Linne (1758) is widely distributed in coastal reef zones of Southeast Asia (Fuze, 1981; Naleewathana and Busarawit, 1988; Singhagraiwan and Sasaki, 1991a, b) extending up to the subtropical and tropical regions of Japan (Hahn, 1989a). Among the two common species of abalone reported in the Philippines (Fuze, 1981), *H. asinina* has the potential for artificial propagation due to its large size and body weight. Recent advances in Thailand have confirmed the potential of this species for seed production and culture (Singhagraiwan and Sasaki, 1991a, b).

A commercial abalone fishery exists in the Philippines. In 1991, the Philippines exported to Hongkong, Japan, United States, Singapore, Australia, and Guam 283, 391 kg of processed abalone worth US $ 2,070,503 (NSO, 1991). Although demand for abalone meat remains high, the status of the fishery, reproductive biology, and seed production trials of *H. asinina* in the Philippines is not known.

Over the last three decades, several commercially important abalone species in several countries have been successfully induced spawn by the use of ultraviolet (uv) irradiation and hydrogen peroxide (Hahn, 1989b). In Japan and U.S., abalone species are induced to spawn on demand throughout the year by the heavily UV-irradiated seawater technique (Uki and Kikuchi, 1984; Ebert and Houk, 1984). Hydrogen peroxide may also induce gravid *H. refescens*, *H. coccinea canariensis*,

and *H. discus hannai* to spawn (Morse et al. 1977; Peña, 1986; Hahn, 1994). However, Singhagraiwan and Sasaki (1991a) failed to induced spawning in *H. asinina* by uv-irradiated seawater; hence, fertilized eggs and larvae were obtained solely from natural spawns.

This study aimed to develop seed production methods of *H. asinina* and to describe its larval development. As successfully carried out in Japan using *H. discus hannai* (Saito, 1984), artificially produced *H. asinina* seed may be used to re-stock coastal areas in the Philippines.

MATERIALS AND METHODS

Broodstock Collection and Maintenance

H. asinina broodstock were collected from shallow rocky reefs of Panagatan Cays, Antique, Philippines (Fig. 1) on January 26 and February 23, 1994. Shell length and total weight ranged from 54 to 108 mm and from 34.6 to 186.8 g, respectively. Broodstock were transported to the Tigbauan Main Station of SEAFDEC/AQD.

Forty-five spawners (10 males and 35 females) were placed in a 1-t oval fiberglass tank. Water inlet and aeration system in the tank were similar to that used at the Oyster Research Institute in Japan (Fig. 2). A polyvinyl chloride (PVC) gutter cut into four 30-cm long sections *ad libitum* to the spawners.

Artificial Spawning

Ripe individuals were selected and placed in separate 9-l rectangular plastic containers. Gonadal maturity was assessed by gently opening a space between the shell and the soft body. Mature males have milky white testis while mature females have dark green ovaries. A gross description of gonad development followed 5 arbitrary stages, depending on the area of the gonad covering the digestive gland. Development varied from immature (Stage 1) to full maturity (Stage 5; Table 1). Only broodstock with gonads at Stages 4 and 5 were stimulated to spawn by dessication (1.0-1.5 h), thermal shock (1.4-5.1°C), UV-irradiated seawater (filtered to 5 µm; 350-1,329 mWh/l), hydrogen peroxide (0.25-1.0 mM), and application of spawned abalone milt (Table 2).

Dessication was carried out by removing individuals from the water and wrapping them in moist sterile gauze for about 1-1.5 h. Thermal shock consisted of raising the water temperature above ambient using a temperature-controlled water heater and then gradually decreasing it to ambient. Artificial induction of spawning using hydrogen peroxide was carried out using reagent grade (30%) chemical. The animals were exposed to the hydrogen peroxide solution for 2-3 h. After this period, the solution was decanted and the container thoroughly rinsed and replaced with clean isothermal seawater. This was not added when hydrogen peroxide was used because it was not required for any specific chemical reaction (Hahn, 1989b). Ultraviolet-irradiation was carried out using 2 uv light systems in series (Toshiba brand; model GWO-1526PB). The amount of uv-irradiation was calculated based on

the flow rate of filtered seawater entering the uv system. Lastly, spawned abalone milt was collected from the broodstock tank released from natural spawning.

Egg Collection and Larval Rearing

Fertilized eggs or trochophore larvae from natural spawnings were collected with a special larval trap (80 µm mesh plankton net) installed at the drain outlet of the broodstock tank (Fig. 2). Collected eggs or larvae were washed with uv-irradiated seawater and then distributed to 9-l rectangular plastic containers.

Trochophore larvae were kept in the same containers until the veliger stage. Upon observation of the operculum, eyespot, and fully formed propodium, veliger larvae (Fig. 4e) were transferred to a 1-t settlement tank provided with vertically placed corrugated diatom plates. Water flow was then stopped for 2-3 d to allow settlement of viable larvae. After settlement, seawater was re-introduced and maintained at a flow rate of 550-600 L/h. Larvae fed on the diatoms *Navicula* sp. and *Nitzschia* sp. that have grown on the plates.

Embryonic, larval, and post-larval development stages were monitored. Morphological characteristics were based from Seki and Kan-no (1977).

Water temperature and salinity ranged from 27.7 to 30.2°C and from 30 to 32 ppt, respectively.

RESULTS

Natural Spawnings

Natural spawnings occurred several days before or during the new moon and full moon (Fig. 3). This trend continued during the initial two months of the experiment and spawnings were observed thereafter to occur for several days after at least every two weeks following a lunar cycle.

Artificial Induction of Spawning

Gravid abalone did not spawn after application of dessication, thermal stock, uv irradiation or hydrogen peroxide. However, milt released by a single male induced 3 females to spawn 1.5 h after milt release. Spawned eggs, however, were immature and few (8.1 -16.2 x 10^3) compared to about 261 x 10^3 eggs obtained from a single natural spawning.

Pumping movement which usually occurs at the time of spawning was observed when hydrogen peroxide was used as spawning stimulus. However, only seawater was discharged from the respiratory pores.

Early development

Newly spawned eggs were green and measured 180 µm in diameter (Fig. 4a). Cleavage began after discharge of the polar bodies and development progressed to

the morula, blastula, and gastrula stages (Table 3). Trochophore larvae hatched 5.2-5.8 h after fertilization (Fig. 4c), began shell secretion, and then transformed to veliger larvae 8 h post-fertilization. At this point, the apical region of the larvae was flat and the velum completely developed with long cilia present. Figure 4d shows a veliger larva before torsion of foot mass while Figure 4e shows a veliger larva after torsion of foot mass with development of operculum, eyespot, propodium, and cephalic tentacle. The veliger larvae acquired creeping ability 30 h after fertilizaton (Fig. 4f) and settled on prepared diatom plates within 2-3 d (Fig. 4g). After 30 d, the first respiratory pore (notch stage) was observed at a shell length of 2.1 mm (Fig. 4h).

DISCUSSION

Artificial Spawning

Dessication or complete removal of individuals from the water, in combination with thermal shock or uv light, was not successful in inducing *H. asinina* to spawn. Dessication alone is unreliable and has no biological significance because abalone are subtidal and would never be subjected to this kind of stimulus in nature, causing even the release of large quantities of immature gametes (Carlisle, 1945; Hahn, 1989b). However, dessication in combination with UV-irradiated seawater yield good results in other abalone species (Uki and Kikuchi, 1984; Chen, 1984; Han *et al.* 1989).

Thermal shock or raising the water temperature above ambient, in combination with various stimuli, also failed to induce spawning in this species. Thermal shock alone, like dessication, is only occasionally successful (Leighton, 1974). It causes the release of immature gametes and does not assure the simultaneous release of viable gametes (Carlisle, 1945; Hahn, 1989b). This technique, however, was sufficient if used during the breeding season of *H. diversicolor supertexta* (Chen, 1984).

Although irradiation of seawater with uv light is a fast and reliable method for induction of spawning in several species of abalone (Uki and Kikuchi, 1984; Ebert and Houk, 1984; Chen, 1984; Han *et al.* 1989; Han, 1989b), it was not effective in inducing *H. asinina* in this experiment to spawn when either singly or in combination with other stimuli. Singhagraiwan and Sasaki (1991a) also were unable to spawn *H. asinina* using uv-irradiated seawater. Spawning failure in this experiment may have been caused by the higher intensity of radiation (including ultraviolet) available in the lower latitude due to unequal distribution of solar radiation on the earth's surface. Tropical abalone species are already exposed to higher amount of uv light than temperate species. Difficulty in assessing fully matured and spawnable individuals by visual inspection and the occurrence of frequent natural spawnings are also other factors to be considered.

Induced spawning of *H. asinina* can be achieved when spawned milt is used to induce gravid females. Similar results were reported by Singhagraiwan and Doi (1992). Adding newly-spawned gametes from either sex into the water can trigger responsive spawning among conspecifics (Carlisle, 1945; Morse *et al.* 1977; Singhagraiwan and Doi, 1992).

Natural spawning

Natural spontaneous spawnings are frequently observed to occur in the present study.

It was noted that *H. asinina* spawns in tanks all year round with a monthly peak in October (Singhagraiwan and Doi, 1992). The same authors also reported partial and multiple spawning of ripe females.

H. varia, another tropical species, also spawns the whole year round as observed in aquaria (Fuze, 1981) and based on observations of gonads from a natural population (Bussarawit *et al.* 1990).

Early development

Embryonic and larval development in *H. asinina* are a function of water temperature and time since abalone larvae do not feed before settlement (Hahn, 1989c). The length of the larval period is about 28.5 h (27.7 - 30.2°C) which is considerably shorter than that for *H. rufescens* with 6 d at 15°C (Ebert and Houk, 1984). Settlement, metamorphosis, and deposition of peristomal shell mark the transition from larval to post-larval development until formation of the first respiratory pore (Hahn, 1989c). Settlement stage was achieved in *H. asinina* after 30 h compared to 168 h for *H. midae* at 20°C (Genade *et al.* 1988). Table 4 shows the age and shell length of *H. asinina* at formation of first respiratory pore compared with other abalone species.

Our results support the strong potential of *H. asinina* as an aquaculture species. However, further studies are required to develop conditioning techniques for captive broodstock (e.g. proper nutrition, appropriate sex ratio, water temperature, water quality, etc.) to allow year round and efficient production of abalone larvae and juveniles.

ACKNOWLEDGEMENT

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Table 1. Criteria used to assess gonad maturation stages in adult *H. asinina*.

Stage	Description of gonad
1	The gonad is essentially immature and difficult to distinguish from the digestive gland; sexes cannot be determined.
2	Developing gonad covering a little portion of the digestive gland; female gonad is still difficult to distinguish from the digestive gland; males can be easily determined.
3	Partially matured gonad; small gonad bulk.
4	Fully matured gonad.
5	Fully matured and swelled gonad; large gonad bulk.

Table 2. Induced spawning trials of *Haliotis asinina*.

Date	No. of Animals	Treatment	Duration or Magnitude	Application Time (h)	Water Temperature (°C)	Response
2/17/94	12 females 3 males	Dessication Thermal shock Thermal shock	1.0 h 28.6 - 30.0°C 28.0 - 30.0°C	1620 - 1720 1750 - 1840 2100 - 2200	27.1 - 28.6	None
3/10/94	3 females 2 males	UV irradiation	1329 mWh/l	1400 - 2200	28.3 - 31.6	None
3/11/94	3 females 2 males	Dessication UV irradiation	1.0 h 925 mWh/l	1700 - 1800 1800 - 0700	27.1 - 28.9	None
3/16/94	3 females 2 males	Dessication UV irradiation	1.5 h 1083 mWh/l	1630 - 1800 1800 - 0900		None
3/17/94	6 females 3 males	Hydrogen peroxide Hydrogen peroxide Hydrogen peroxide	0.26 mM 0.50 mM add 0.50 mM	1000 - 1300 1300 - 1600 1600 - 1800	28.8 - 29.8	None
3/22/94	3 males	Hydrogen peroxide	0.50 mM	1440 - 1700		None
3/23/94	2 females 3 males	Hydrogen peroxide Thermal shock Thermal shock	0.25 mM 28.9 - 34.0°C 33.2 - 35.6°C	1053 - 1300 1100 - 1111 1207 - 1213	28.9 - 31.6	None
3/24/94	15 females 5 males	UV irradiation Dessication Thermal shock Spawned milt	350mWh/l 1.3 h 29.2 - 32.0°C 240,000 sperm/ml	1820 - 1940 2020 - 2040 0330	27.7 - 29.6	3 females spawned

Table 3. Embryonic and larval stages of Haliotis asinina reared at 27.7 - 30.2°C.

Stage	Time after fertilization (h)
Fertilization	0
First cleavage	—
Second cleavage	0.9
Third cleavage	—
Morula stage	1.2
Blastula stage	—
Gastrula stage	1.5
Trochophore larva	4.0
Hatch-out	5.2 - 5.6
Shell secretion	6.0
Veliger larvae	
Larval shell formulation	6.0
Larval shell completion	9.0
Operculum formation; rotation of footmass	13.0
Eyespot formation	19.0
Appearance of otolith; creeping ability	30.0
Juvenile	
First respiratory pore	30 d

Table 4 Age and shell length of various abalone species at formation of first respiratory pore

Species	Age	Shell length (mm)	Temperature (°C)	Reference
H. asinina	30	21	27.7 30.2	Present study
	28	20	29 30	Singhagraiwan and Sasak (1991a)
H. corrugata	50 60	20 25	15 22	Leighton (1974)
H. fulgens	30 40	17 20	16 24	Leighton (1974)
H. rufescens	60 70	15 18	14 18	Leighton (1974)
H. sorensani	55 65	20 21	14 18	Leighton (1974)
H. midae	48	23	17 22	Genade *et al.* (1988)
	65	23	12 17	Genade *et al.* (1988)

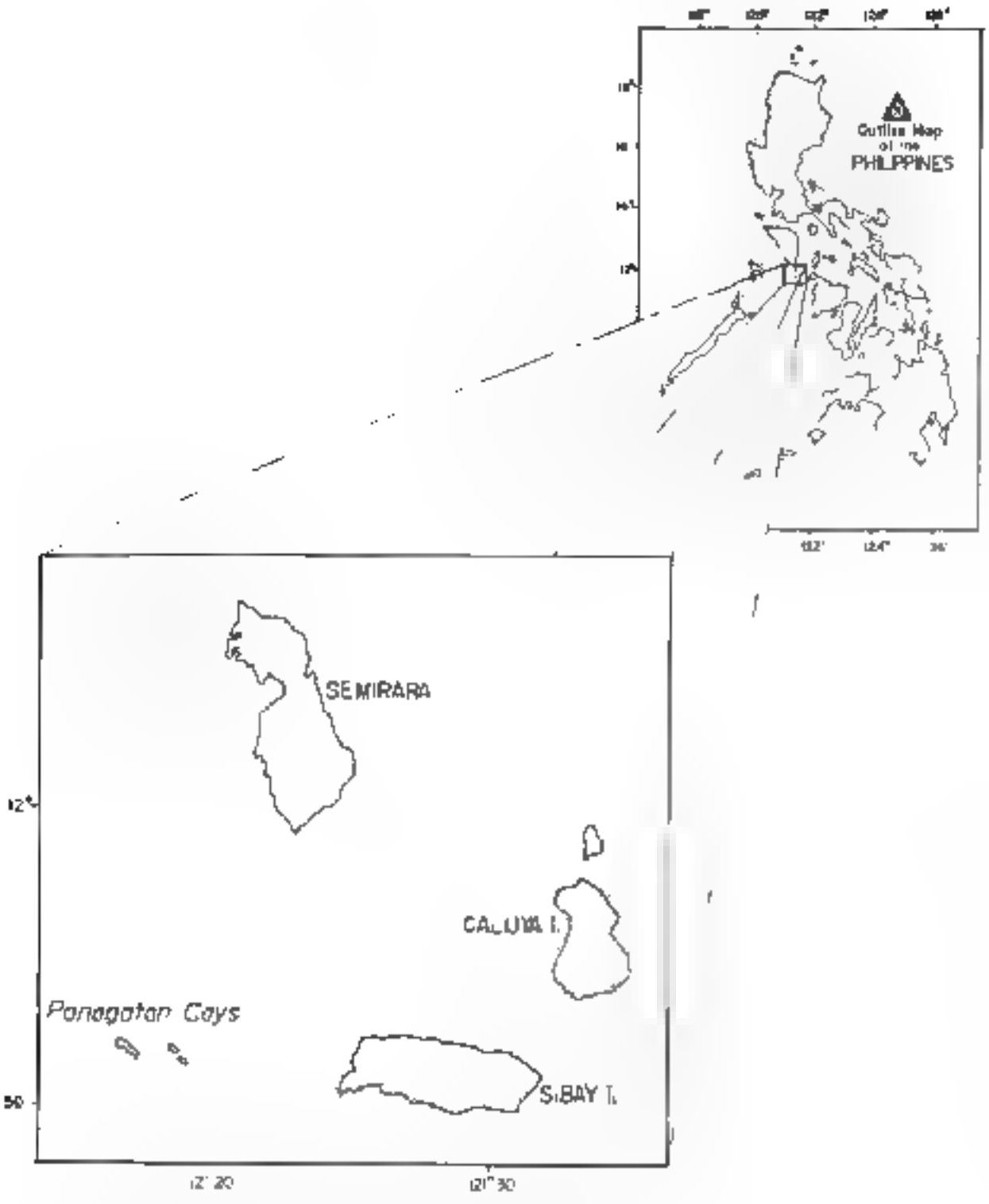

Figure 1. Map showing the location of Panagatan Cays, Antique in Central Philippines.

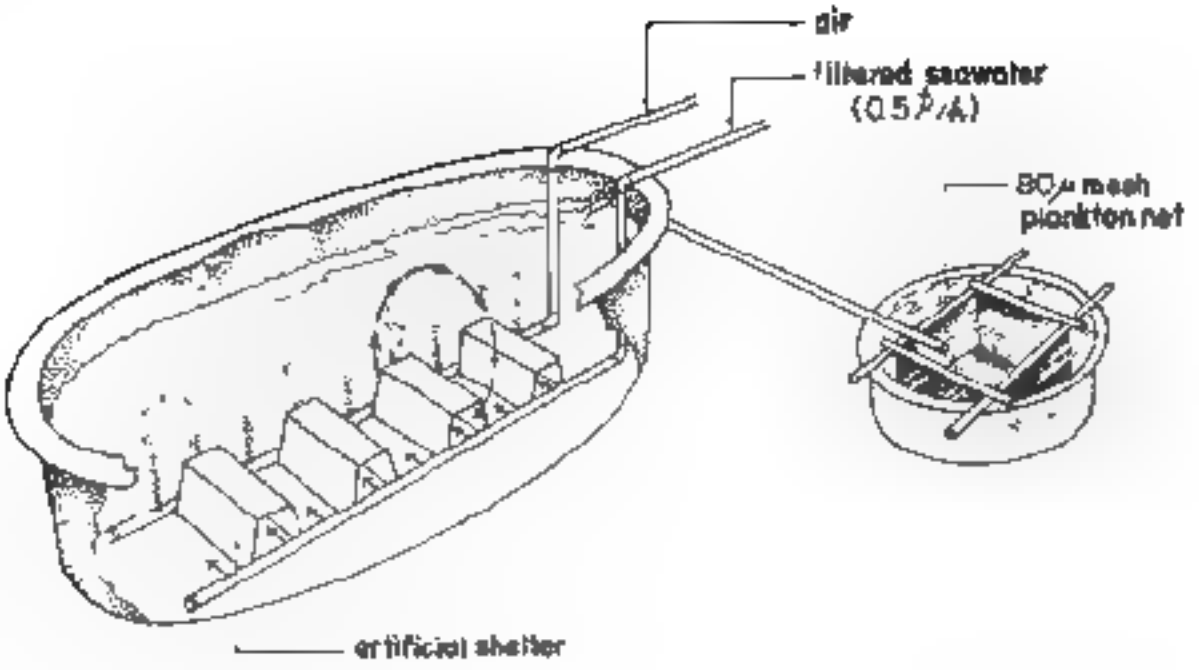

Figure 2 Broodstock conditioning tank for *H. asinina* with special larval trap for egg/trochophore larvae collection.

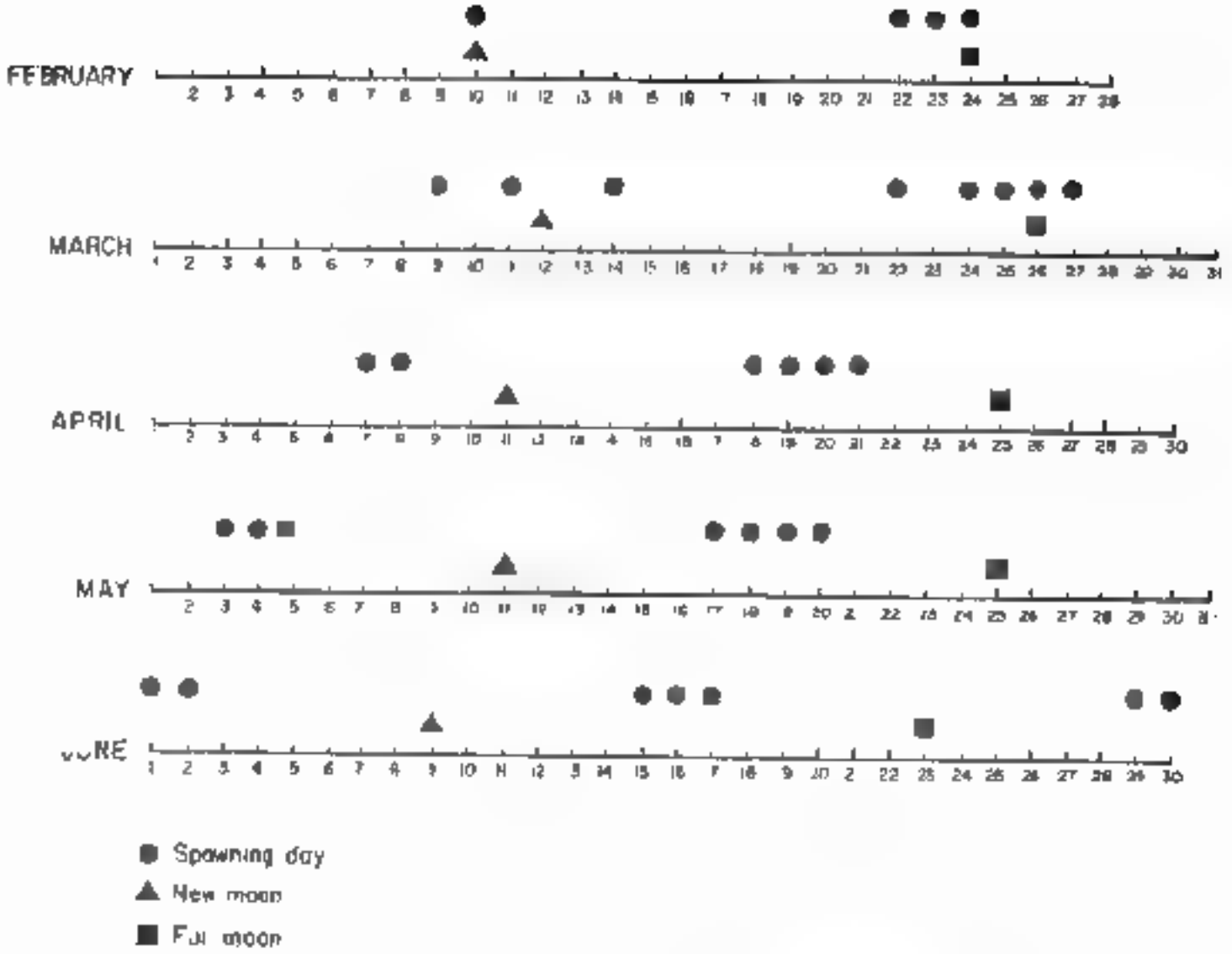

Figure 3. Natural spawnings observed in broodstock tank from February to June 1994.

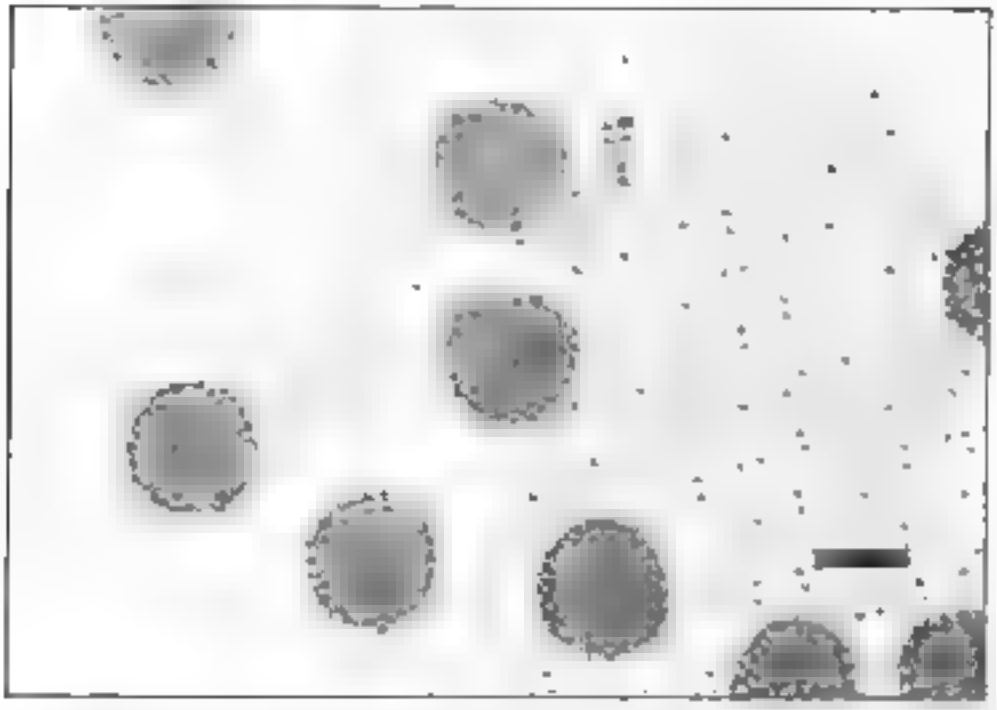

Figure 4a. Developmental stages of *H. asinina* at 27 7 30.2°C A fertilized eggs (180 μm)

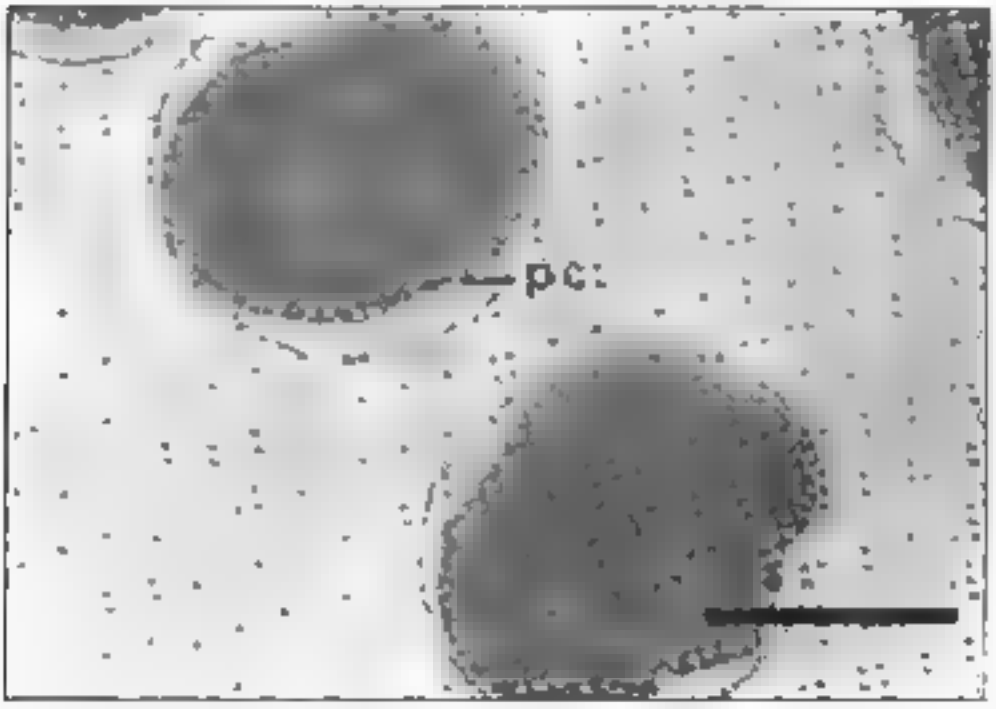

Figure 4b Developmental stages of *H. asinina* at 27 7-30 2°C Trochophore stage before hatch-out (4.5 h after fertilization pc prototrochal cells).

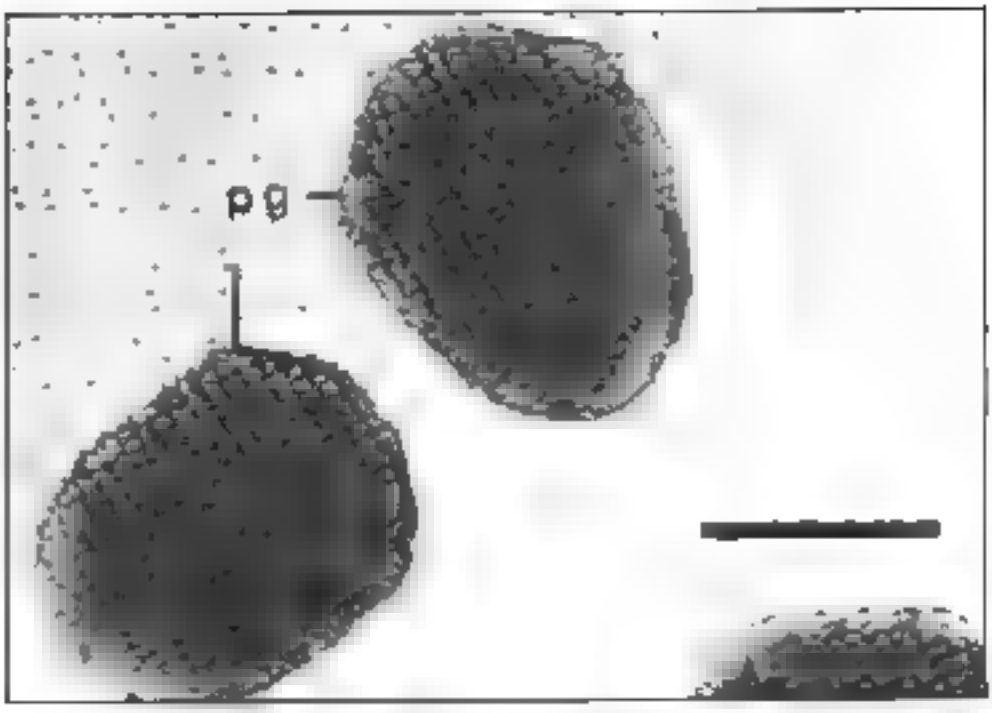

Figure 4c. Developmental stages of *H. asinina* at 27.7-30.2°C. Trochophore stage after hatch-out (pg, prototrochal girdle).

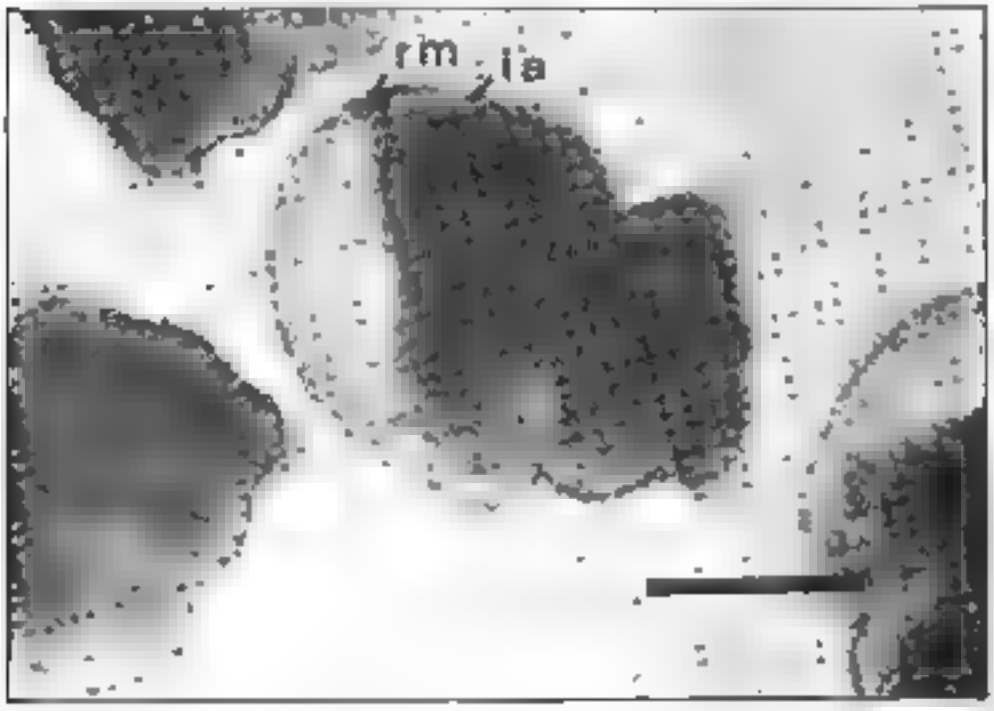

Figure 4d. Developmental stages of *H. asinina* at 27.7-30.2°C: Veliger larva at completion of larval shell and before torsion of footmass (rm, retractor muscle; ia, integumental attachment).

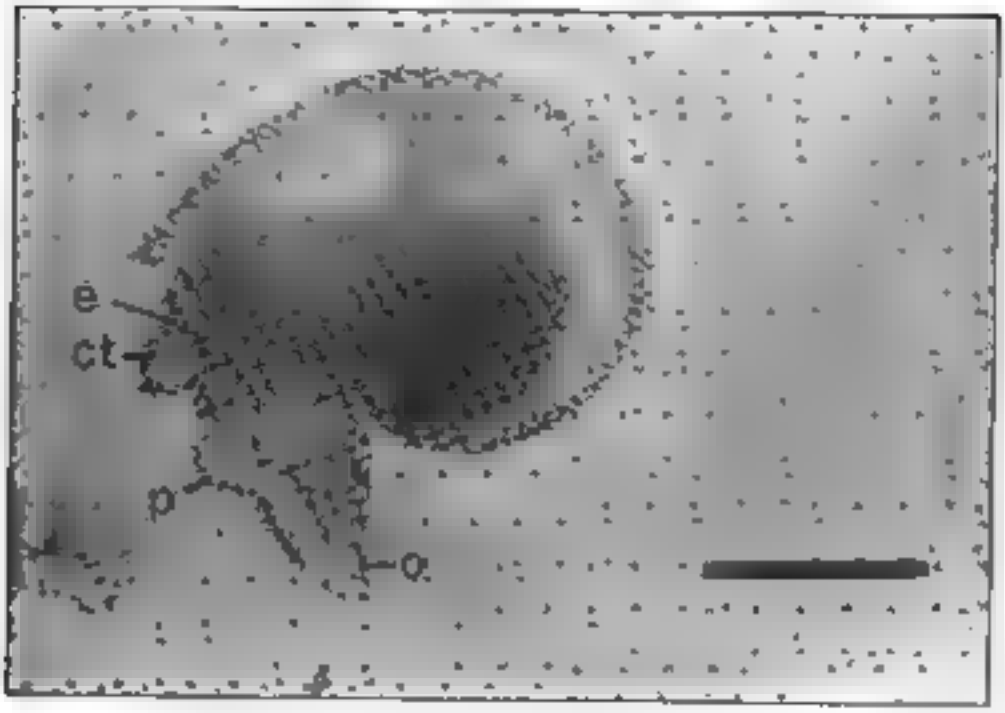

Figure 4e Developmental stages of *H. asinina* at 27.7-30.2°C. Ve iger larva after torsion of footmass (o, operculum; e, eyespot; p, propodium; ct, cephalic tentacle)

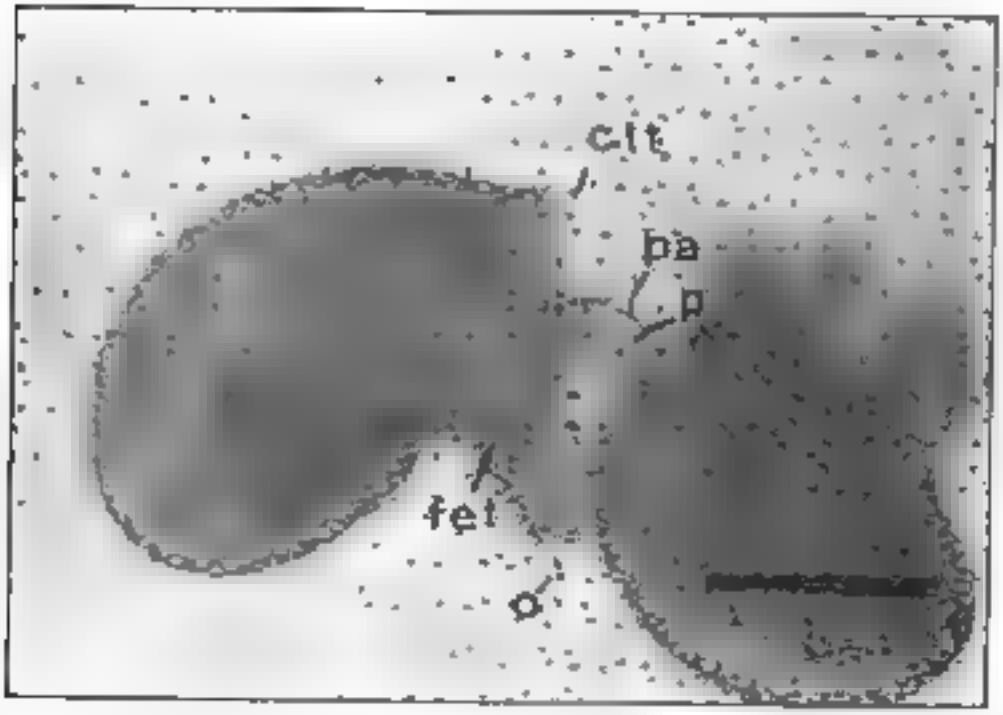

Figure 4f. Developmental stages of *H. asinina* at 27.7-30.2°C. Veliger larva before settlement (30h after fertilization, pa, propodium apophysis; fep, first epipodial tentacle)

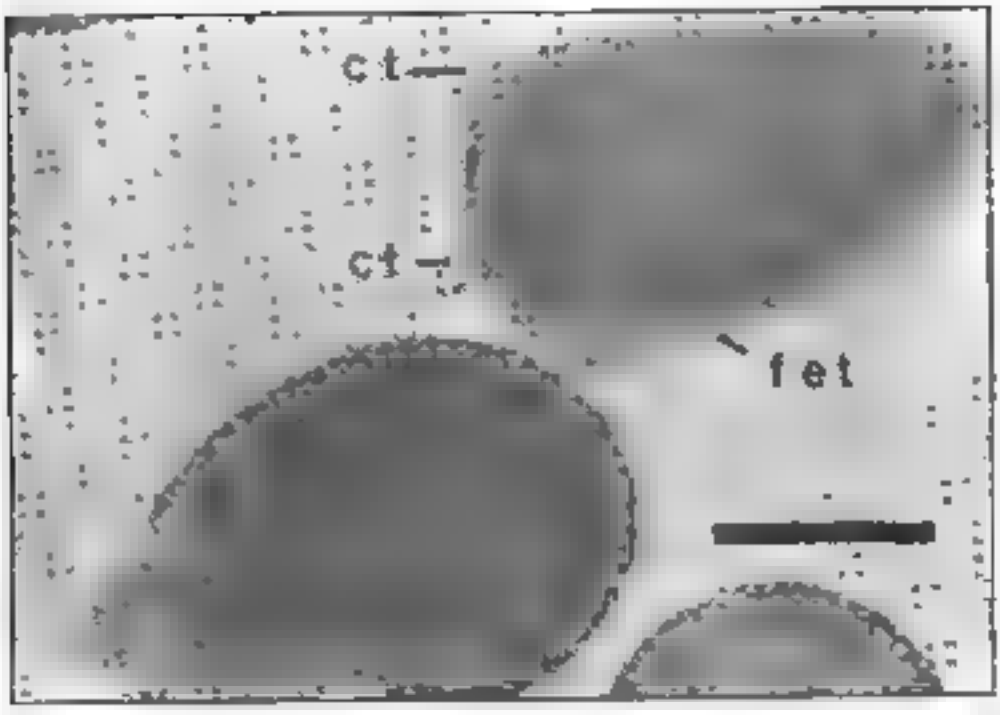

Figure 4g. Developmental stages of *H. asinina* at 27.7-30.2°C. Creeping larva (ct, cephalic tentacle; fep, first epipodial tentacle).

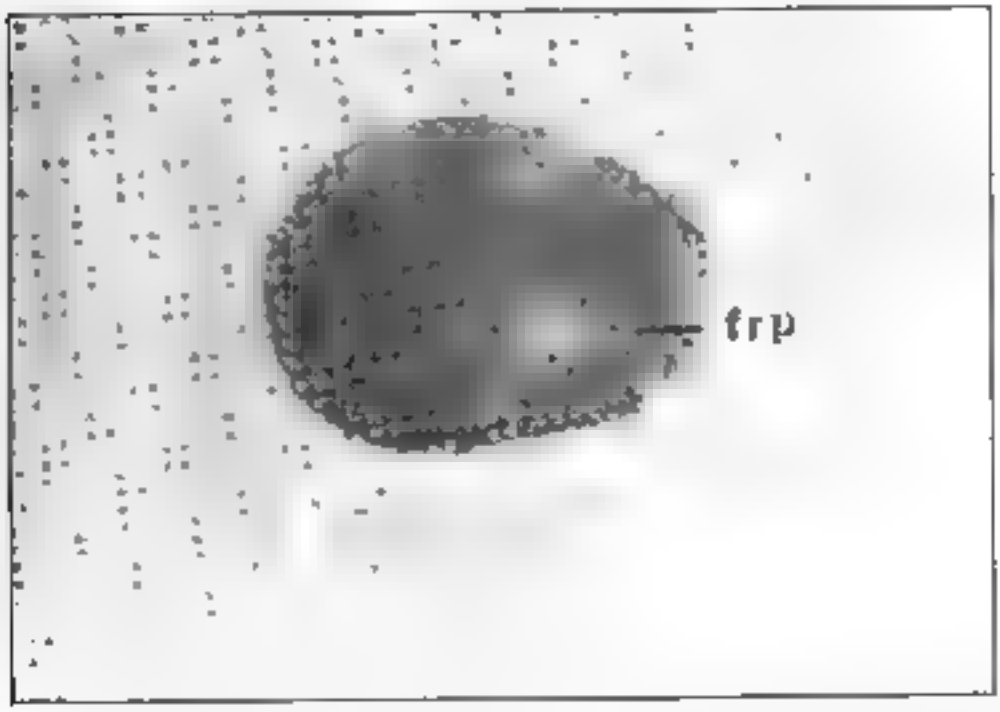

Figure 4h Developmental stages of *H. asinina* at 27.7-30.2°C. Juvenile with first respiratory pore (30 days at a size of 2.1 mm). Scale bar = 100µm

INFLUENZA AS ZOONOSIS
AN OVERVIEW

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ABSTRACT

[illegible] including the appropriate [illegible] for the current season/ Extensive [illegible]

History and epidemiology

[illegible]

Antigenic drift and antigenic shift

in more or less periodic intervals

A unique zoonosis

[illegible] is not uncommon in ducks, quail and turkeys.[illegible]

[illegible] circulating human influenza viruses.[illegible]

[illegible]

in 1918 in north central United States during the annual winter outbreaks. Epidemiologic observation suggested that the viruses were introduced into the swine population from strains then circulating among man. The swine influenza virus implicated in this epidemic is believed to be related to the 1930 isolate by Shope, the first time the influenza virus was isolated in swine. In the period between 1918 to 1930, the only laboratory evidence for the circulation among humans of viruses related to swine influenza was the detection of high titres of naturally occurring antibodies to swine influenza viruses in the sera of persons who were alive during the period of the influenza epidemic.[8] Other than the classic H1N1 variants, the avian-like H1N1 and the human-like H3N2 are concurrently circulating in pigs worldwide.[44-46]

A typical outbreak of swine influenza is characterized by sudden onset and rapid spread through the entire herd, often within 1 to 3 days. The main signs are depression, fever, anorexia, coughing, dyspnea, muscular weakness, prostration, and a mucous discharge from the eyes and nose.[1]

In 1974, the isolation of the first human influenza virus similar to swine influenza came from a young patient with Hodgkin's disease who lived in a farm and had contact with pigs. In February 1976, outbreaks of swine influenza-like illnesses among persons who had no direct contact with pigs at that time were reported in Fort Dix, New Jersey. It was believed that the unique environment in Fort Dix facilitated transmission from person to person of swine influenza virus after its first introduction by someone who did contract an infection by exposure to an infected pig.[8]

The swine influenza virus is also known to infect birds, principally turkeys.[4] One American study demonstrated that 23% of the turkey influenza virus isolated contained genes of swine origin. Castrucci and co-workers study provided the first evidence supporting genetic reassortment between human and avian viruses in a natural swine environment.[1] Another study furnished evidence that the antigenic determinants on H3N2 influenza viruses were conserved in pigs by reassortment of avian and human H3N2 viruses.[10] The economic consequences of swine influenza are considerable in that sick pigs lose weight and their weight gains are often reduced. However, the real public health significance of pigs in influenza lies in their probability to act as mixing vessels for genetic reassortment of the influenza viruses from other species.

Equine influenza is widely distributed, highly contagious and spread rapidly. The rapid international spread is due to the year round transport of horses for racing and breeding purposes especially in Western Europe and North America. The severity of the illness varies with the presence of other non influenza pathogens.[11] The isolation of the first equine influenza viruses, A/equi 1/Prague/56 (H7N7), was from an epidemic in Europe in 1956. In 1963, influenza A viruses, A/equi 2/Miami/63 (H3N8), having no antigenic relationship in their H or N to those of previously circulating equine strains were recognized in the U.S. Both continue to circulate without evidence of naturally occurring recombination. Although horses are the only known reservoir of equine influenza viruses, it is now known that the H and N antigens of the two equine subtypes are related, although not identical, to antigens found among human, avian, or swine influenza A viruses. The H7 of

subtype 1 is related to some strains of the fowl plague viruses, and the H3 N subtype 2 to some human and some avian H3 strains. Some examples of highly similar influenza A viruses are: A/Hong Kong/1/68 (H3N2), A/equi 2/Miami/1/63, A/duck/Ukraine/1/63 (H3N8) and A/swine/Taiwan/7310/70 (H3N2).[7]

The onset of equine influenza is abrupt, with temperature up to 42°C, usually lasting less than 3 days unless bacterial infection follows. Coughing is observed early and may persist for several weeks. Nasal discharge is scant. Expiratory dyspnea, anorexia, weakness and stiffness are sometimes present. Mildly affected horses recover spontaneously within 2 to 3 weeks, but those severely affected may convalesce for 6 months.

Other mammalian species have more rarely been reported to be the source of influenza A viruses and increased surveillance of animals over the past years has identified only a small number of new subtypes of influenza H or N antigens. Investigations had strongly suggested that influenza A subtype H3N2 viruses can naturally infect dogs, but the rate of seropositives is too low to consider dogs as potential reservoir of this subtype.[8]

Detailed investigations provide evidence that the genetic evolution of the influenza viruses is quite rapid.[9,10] This property allows fast adaptation of the virus to the changing environment, thus the emergence of the numerous variants. The reassortment and recombination of the variants give rise to a genetic pool that is shared by susceptible species notably man, birds, pigs and possibly horses making influenza a unique zoonosis compared with other zoonoses where a single specific pathogen can directly infect several species.

Surveillance

The World Health Organization's (WHO's) program on laboratory influenza surveillance is based on the collaboration of 110 national institutes for influenza in 77 countries which are in contact with the WHO headquarters in Geneva and the 2 WHO Collaborating Centres on Influenza Reference and Research in Atlanta, USA and London, England. The network of national institutes covers all parts of the world: 46 laboratories are located in 25 developed countries and 64 laboratories are in 52 other countries. Isolates of influenza viruses obtained by the national institutes are sent to the two WHO Collaborating Centres for analysis of all antigenic peculiarities of hemagglutinin and in some cases of neuraminidase.

In Geneva, all epidemiological and laboratory information is consolidated and published regularly in the *Weekly Epidemiological Record*, which is widely distributed to health authorities, influenza centers, and other interested institutions and persons. Each year, at the end of February, the WHO holds a meeting with the directors of the WHO influenza centres to decide upon the composition of the influenza vaccines for the forthcoming epidemic season. The resulting recommendations, which are published in the *Weekly Epidemiological Record* at about the end of February, are based on the various information sources, including epidemiological data, serum surveys, data from vaccine trials and the results of studies on antigenic characteristics of influenza viruses isolated in different countries.[3]

CONCLUSION

Based on recent findings and developments, as well as on the theory of "recycling" of the influenza A viruses (refer to Figure 1), there are strong indications that the next pandemic would of the subtype H2N2[38,50-51]. In the Philippines there is a dearth of information on animal influenza and no laboratory surveillance in human influenza. It would be useful to conduct a seroepidemiological study using the hemagglutination-inhibition test on the influenza virus in the human, avian, porcine and equine populations in the country. Later characterization of isolated local strains by polymerase chain reaction or other methods may help trace the evolution of the pandemic subtypes of the virus, especially the Asian strains. Once the ecological properties of influenza viruses are understood, it may be possible to interdict the introduction of new influenza viruses into humans. Vigilant virus surveillance would help in anticipating a future pandemic.

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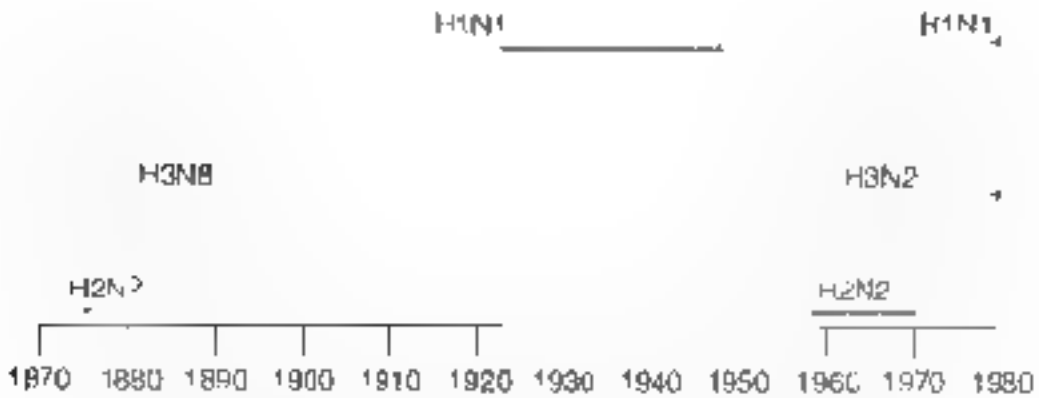
, Determined by serum antibody prevalence
dates of appearance are unknown and of disappearance inexact

, Approximate birthdate of cohort with highest antibody prevalence.

Determined by virus isolation



Source Concepts and procedures for laboratory-based influenza surveillance (WHO 1982)

Figure 1 Eras of prevalence of human influenza A viruses with 1980 WHO nomenclature for H and N subtypes.

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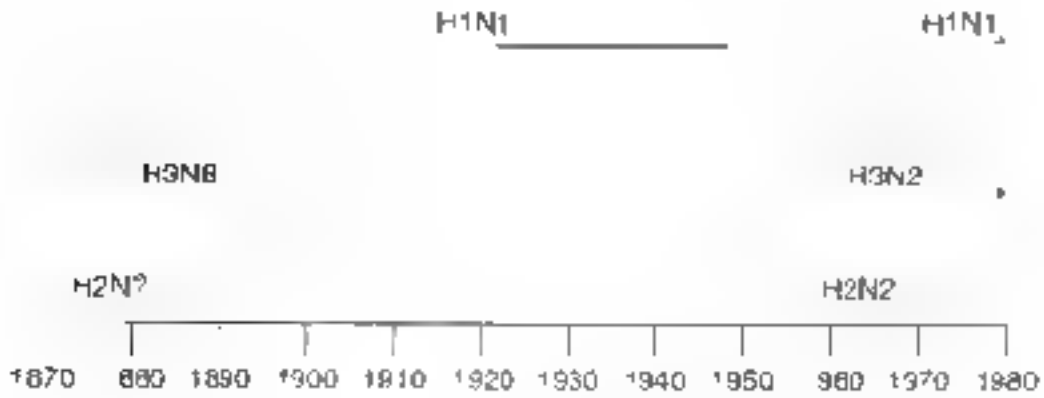
Approximate birthdate of cohort with highest antibody prevalence

Determined by virus isolation



Source Concepts and procedures for laboratory-based influenza surveillance (WHO. 1982)

Figure 1 Eras of prevalence of human influenza A viruses, with 1980 WHO nomenclature for H and N subtypes.

SORPTION BEHAVIOUR OF LICORICE

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ABSTRACT

Adsorption isotherms of licorice in two forms viz. root and powder were determined. Equilibrium Relative Humidity was 65% at initial moisture content of [illegible]% at [illegible]°C. Licorice in root and powder forms were found to store well when the moisture content was below [illegible]% and [illegible]% respectively.

INTRODUCTION

A food moisture sorption isotherm describes the relationship between the moisture content in food and the Relative Humidity (RH) of the air with which the food is in equilibrium at constant temperature and pressure (Labuza, 1968). Therefore moisture is used as an important factor indicating the adequacy of food and food products for storage. If however they are not properly packaged moisture content may change in relation to the environmental RH through dehydration or hydration [illegible]. A short period exposure of the dried stored products to a highly humid atmosphere would proceed rapidly to the germination and growth of the fungal spores. This growth is inevitable even if the moisture content of the product is regarded as safe (Troller & Christian, [illegible]).

Licorice (*Glycyrrhiza glabra* [illegible]) extract is one of the oldest plant extracts used in most of the national pharmacopoeias and formularies (Adams, [illegible]). It may be added in smaller proportions as a flavouring substance in confectionary products such as chewing gums, caramels, fudge, toffees etc. [illegible] confectionary is distinguished on the basis of manufacturing process as cast moulded licorice and extruded (pressed) licorice. The extract is obtained from the licorice root and the yield is about [illegible] 40% of the root. However a wide variation in the yield due to various factors is reported (Sag[illegible], 1954).

In India it is normally grown in sub-tropical region and after harvesting, air dried licorice is stored at various places in conventional jute bags. As a result the fungal contamination due to variation in the humidity and moisture content is observed. The fungal growth significantly affected the important properties such as flavouring, [illegible] and percent of yield. At present there is no specific report available on the optimum moisture content, humidity and storage conditions required for the storage of licorice. Therefore in this study an attempt was made to investigate fairly and accurately the moisture content and water activity of licorice to protect from fungal contamination.

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MATERIALS AND METHODS

For this study, the sorption behavior and keeping quality of [illegible] in its [illegible] form [illegible] grain form powder was considered. [illegible] that was purchased from [illegible] market [illegible] samples were discarded. It was powdered in commercially available [illegible] grinder and passed through a [illegible] sieve. The powder so obtained was filled in an air tight glass bottle with glass stopper and stored in a refrigerator at [illegible].

Moisture equilibrium studies

Sorption studies were carried out according to static method (Greenspan, 19[illegible]; Rockland, [illegible]). Changes in the samples weight due to gain or loss of moisture content was [illegible] at 24 h intervals [illegible] equilibrium. Moisture content (EMC) of each of the [illegible] was computed on dry weight basis. [illegible] equilibrium relative humidity (ERH) [illegible] moisture content of the samples were measured by [illegible] NOVASINA [illegible] measuring system (NOVASINA AG, Switzerland). Changes in colour and general condition of the samples were also recorded.

RESULTS AND DISCUSSION

[illegible] was 43% at initial moisture content of 8.7[illegible]%. At the beginning of the experiment an inconsistency in gain or loss of moisture was observed due to [illegible] surface of the samples (Halsey, 1948). However, equilibrium was attained in all samples within few days. The gain or loss in moisture content is given in Table 1. After equilibrium no gain or loss in moisture content of the samples was observed. The adsorption isotherms of [illegible] and powder [illegible] at room temperature are shown in Figure [illegible]. The isotherms are sigmoid and [illegible] [illegible] classification of Brunauer et al. [illegible] et al. [illegible]. The changes in moisture content and quality characteristics of [illegible] and powder [illegible] conditions are presented in Table 2. The [illegible] was good and acceptable [illegible] and powder form was good and acceptable [illegible]. Above [illegible] RH, the powder sample became lump with the loss of free flowiness and was not acceptable. Mold growth was observed at [illegible] RH [illegible] day and at [illegible] in powder on [illegible] day. The colour of the samples became pale bluish green due to the growth of fungus *Aspergillus niger*.

Sorption isotherm shown in Figure [illegible] indicates sharp or steep rise in the isotherms above [illegible] RH for [illegible] and above [illegible] RH for powder. The beginning of the steep rise in the sorption isotherm is generally recognised as an indication of the onset of rapid physicochemical and microbiological deterioration in the product. As such, the moisture contents of 1[illegible]% in [illegible] and 1[illegible]% in powder corresponding to [illegible] and [illegible] RH are considered to be safe and moisture above this critical level will lead to rapid deterioration in respective of samples studied.

The [illegible] of packaging systems consisting of high barrier films is therefore necessary to obtain extended shelf life.

ACKNOWLEDGMENT

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Table 1 Changes in Moisture Content of LICORICE during Equilibration

DAY	Moisture gain/loss (%) in each day at different RHs (%)								
	22	32	43	52	65	69	75	80	85
1	0.1316	0.1639	0.1841	0.2317	0.2912	0.4664	0.4712	1.0216	1.2010
2	0.0924	0.0771	-0.0326	0.1395	-0.0144	0.0416	0.2462	0.5358	0.3731
3	0.0632	-0.1332	-0.0401	-0.0524	-0.1254	-0.2205	1.4516	1.0340	1.1147
4	0.0687	0.2146	0.3267	0.0782	0.2558	0.3391	0.2224	1.1099	1.4199
5	0.0229	0.0928	0.1293	0.2012	0.3271	0.4944	1.5114	0.7358	1.3353
6	0.0.96	0.1521	0.1941	0.4280	0.3077	0.3222	0.5135	1.2975	1.1940
7	±0.0000	0.2343	0.1301	0.1772	0.3540	0.3542	1.6199	0.4669	0.8244
8	±0.0000	0.2026	0.2742	0.6480	0.5425	0.4269	0.3154	0.7900	0.6189
9	–	±0.0000	±0.0000	±0.0000	0.7495	0.8257	0.2977	1.1188	0.3233
10	–	±0.0000	±0.0000	±0.0000	±0.0000	±0.0000	±0.0000	±0.0000	±0.0000
1	0.2841	0.3428	-0.3806	1.5409	1.6831	1.9016	2.0668	2.8114	2.9472
2	1.5433	1.6853	0.7404	1.2265	1.4021	1.1045	-0.5778	1.2344	1.1591
3	0.5743	0.6610	-0.3295	1.4516	1.8896	0.6774	1.1098	1.1661	1.2821
4	1.1655	0.5619	-0.0213	1.0165	1.2067	1.8720	1.9178	2.7306	2.5625
5	0.4076	0.3596	1.3416	-0.0455	-0.0245	1.3235	1.7852	1.2928	2.1409
6	±0.0000	0.6207	0.8844	-0.0431	-0.0328	1.6262	1.1477	2.6817	1.9574
7	±0.0000	±0.0000	1.7244	-0.0424	-0.0131	1.2210	1.1410	1.2519	1.1813
8	–	±0.0000	±0.0000	±0.0000	-0.0207	0.4229	1.0727	1.6693	2.6632
9	–	–	±0.0000	±0.0000	±0.0000	±0.0000	±0.0000	1.2526	1.5585
10	–	–	–	–	±0.0000	±0.0000	±0.0000	±0.0000	±0.0000

* Values indicate the average of two determinations

Table 2. Moisture Humidity Relationship of Licorice at 28±4°C.

RH (%)	The Day Equilibrium Attained		EMC (%)	
	ROOT	POWDER	ROOT	POWDER
22	6th	5th	9.12	12.70
32	8th	6th	9.73	12.96
43	8th	7th	9.89	13.45
52	8th	7th	10.58	13.83
65	9th	8th	11.41	14.82
69	9th	8th	11.72	15.32
75	9th	8th	15.37	16.17 *
80	9th	8th	16.94 *	20.02 *
85	8th	8th	17.13	21.30 *
87	4th	Could not equilibrate - Oscillating*		
92	4th			

Mould growth during equilibration process and visually observed on the day

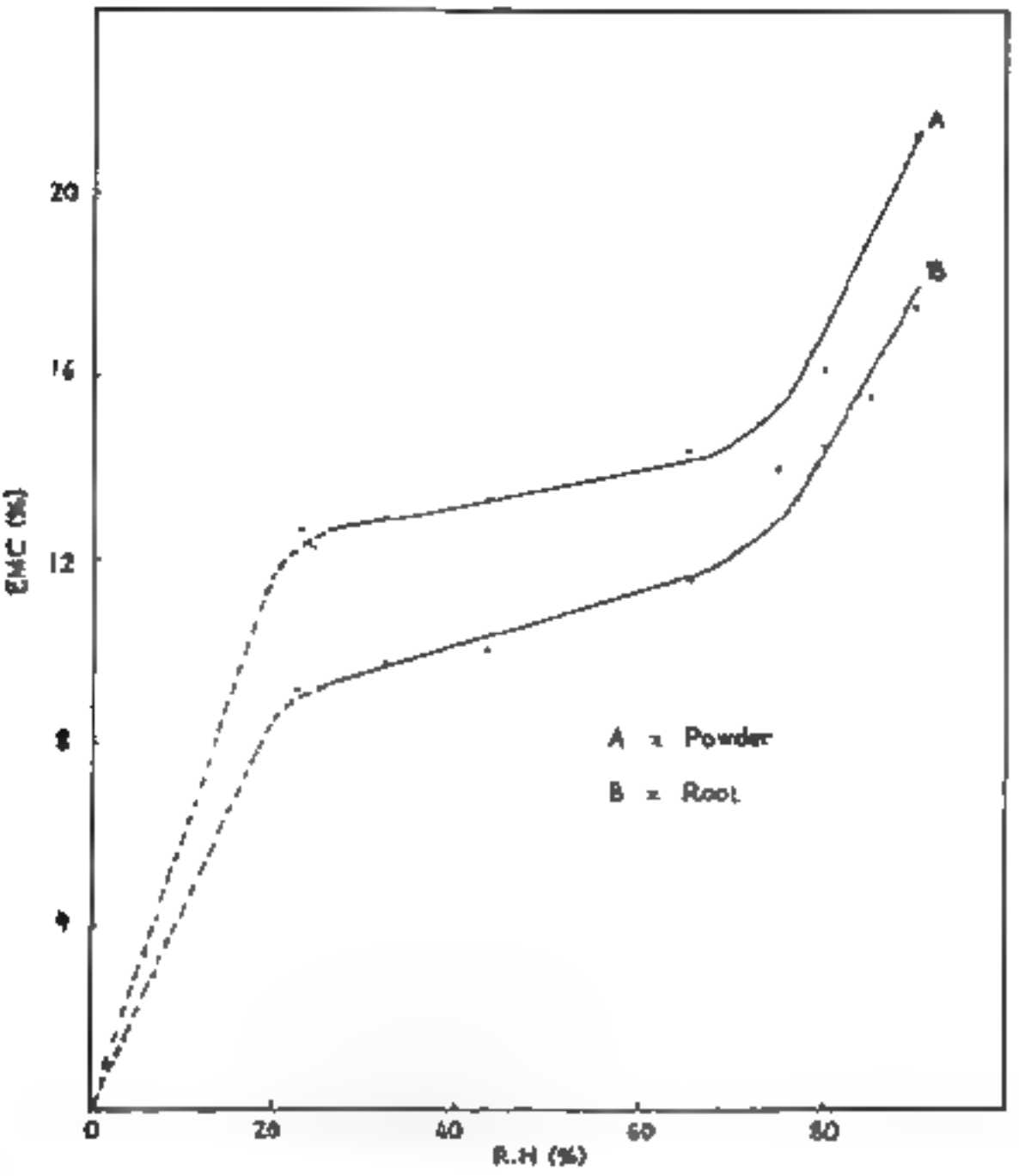

Figure 1 Humidity - Moisture Equilibrium Curves for Licorice Root and Powder.

FOOD ENERGY EQUIVALENTS OF SOME COMMON PHYSICAL ACTIVITIES

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ABSTRACT

The study aimed to develop a table on food energy values in terms of energy equivalents of some common physical activities. The energy value of a specific food was reported in terms of equivalents of time spent in performing these activities. [illegible] healthy male and female adults were selected by [illegible] sampling from employees of Food and Nutrition Research Institute (FNRI) and nearby government agencies who met the inclusion criteria set for the study. [illegible] while performing activities such as sitting, [illegible] walking, jogging, cycling, [illegible]. The energy cost of these activities were measured through indirect [illegible] analyzer. The energy values of some popular food items were derived from the [illegible] Energy Counter and Philippine Food Composition Tables. The mean metabolic costs for all the activities were higher for the male group. Jogging was the most physically demanding, burning [illegible] kcal/min for men and [illegible] kcal for women, followed by cycling and brisk walking, while sitting gave the lowest value. The activity time equivalent of each food was computed using the formula: [illegible] energy cost/minute

INTRODUCTION

Physical activity has for many years been promoted as a general health measure. [illegible] performance of a particular activity.

Based on the statistics of the Department of Health, there is an increasing trend in the incidence of heart diseases [illegible] daily physical activity.

This study provides a table of the energy values of some popular foods among Filipinos, matched with the corresponding length of physical activities required to burn them off. This table will help doctors, nutritionists, dietitians, dieters and weight conscious individuals in planning a weight maintenance/reduction program. While a similar study was done by Konishi (Konishi 1985), results were based on foreign values and foreign food items so that their use in the Philippine setting will be limited.

METHODS

Seventy subjects, 35 males and 35 females, were selected by sequential sampling based on the following criteria:

1. Age between 20-40 years old;
2. Body weight within normal Filipino standards (FNRI-DOST 1975);
3. Absence of illness of the thyroid glands, heart and lungs;
4. Employed in member agencies of the Manila Health Science Community; and
5. Willingness to participate in the study.

The activities measured were jogging, cycling, brisk walking, stretching and flexing, leisure walking (or walking at own pace) and sitting. The energy cost of the different activities was measured by indirect calorimetry using the Douglas bag assembly apparatus while expired air was analyzed using the Servomex oxygen analyzer. Each subject was tested for eight minutes during sitting activity and three minutes each for the strenuous activities. Duplicate samples were taken for all six activities. A stationary bicycle was used for the cycling activity. The speed was fixed at 20 km/hr without any load which simulated the average bicycle ride. In the brisk walking activity, subjects were asked to walk the fastest sustainable pace without actually running. The stretching exercise consisted of common warm up and bending exercise for the neck, arms, waist, hips and legs.

The energy costs of the different activities were calculated by adapting the regression analysis where x = weight or independent variable and y = estimated energy cost or dependent variable. Moreover, the reference body weight of 56 and 49 kg for the average Filipino adult man and women respectively (FNRI-DOST 1989) were used in the calculation of the energy costs of the different activities included in the table.

The food items selected include those that are considered to be the most popular among the Filipinos. The energy value of the food items in the table were derived from the publications "Energy Counter" (Guzman 1979) and the Philippine Food Composition Tables (FNRI-NSDB 1990). Food items were given in common measures such as tablespoon, cup, retail pack or in estimated amounts normally consumed in one sitting. The edible portion was derived by subtracting the non-edible part or the refuse from the total weight of the food.

The calorie time equivalent for each item was derived using the formula calories in the food / energy cost/minute

RESULTS AND DISCUSSION

Physical Characteristics

The summary of data on physical characteristics of the subjects are presented in Table 1. It shows the mean age, height, weight, body mass index (BMI) for both sexes. As expected, the mean height and weight of the male subjects were greater than their female counterparts. However, the mean age and BMI were greater for the females than the male subjects.

Food Items

Foods were classified according to the major group to which they belong. The approximate measure of each food item, its weight in grams, caloric content and energy equivalent in minutes, are shown in Appendix I.

Energy Cost of Activities

The energy cost of the aforementioned activities, i.e., walking, brisk walking, jogging, biking, stretching and flexing, expressed in kcal/min are presented in Table 2.

Obviously, the mean metabolic costs for all the activities were higher for the male group. Jogging was found to be the most physically demanding at 6.05 kcal/min for men and 4.69 kcal/min for women. Cycling came next, then brisk walking, while expectedly, sitting was the easiest and least strenuous. Therefore, jogging would require the least number of minutes to burn off a food item, while sitting would take the longest time to burn the same. Males have higher metabolic costs than females due to a number of factors. Firstly, males are inherently bigger and stronger. Thus can perform strenuous activities with greater intensity than females. Secondly, males, in general, have a more developed and better defined musculature and possess lesser body fat than females. Their muscles, bigger and stronger, require more calories to maintain the daily physical labor subjected on them (Kuntzleman, 1979).

As a whole, the energy cost of the activities tested in this study were within the range stated by Durnin and Passmore (Durnin, 1967).

SUMMARY AND CONCLUSION

The study was conducted to develop a table of energy values of some popular local food items, matched with the corresponding length of physical activities required to burn them off. Among the various activities measured, jogging gave the highest energy cost, followed by cycling and brisk walking, while sitting gave the lowest value.

This study can be of considerable help to people especially those whose lives depend on the healthy balance between the food they eat and the amount of exercise needed to expend it. It will increase the awareness of Filipinos of keeping the body physically fit to prevent diseases.

It must be emphasized that the findings were calculated by setting a reference body weight of 49 kg for women and 56 kg for men. In addition, reference speed and intensity for walking and jogging were not established, thus varying from one individual to another. Some can walk faster than others while others jog with the intensity and speed of an athlete in training. Aside from the body weight, intensity and speed, there are still other factors to consider which may influence the metabolic cost of activities such as the type of surface, grade or level of surface, type of clothing and the weight of the shoes.

Nonetheless, the energy equivalent table provides vital information on energy expenditure and its relation to energy intake. It is therefore desired that people become more aware of the role they play in maintaining their own good health and the importance of daily physical regimen in a rapidly automating environment.

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WEIGHT-FOR-HEIGHT FOR FILIPINOS (25-65 years) Biomedical Nutrition Division, FNRI-DOST

Table 1 Physical Characteristics of the subjects (x ± SD).

Sex	Age	Height	Weight	BMI
Male	27.83 ± 4.99	164.78 ± 4.73	57.74 ± 6.20	21.24 ± 1.77
Female	33.51 ± 4.30	155.09 ± 5.29	51.55 ± 5.03	21.42 ± 1.15

Table 2. Energy costs (kcal/min) of the different activities

Activity	Male	Female
Sitting	1.41	1.10
Flexing and Stretching	3.05	2.53
Walking at own pace	3.25	2.47
Brisk walking	4.99	3.59
Cycling	5.04	4.12
Jogging	6.05	4.69

Annotation to the chart

The chart (see next page) lists the most common food items, their caloric content and their energy-time equivalent serving as a guide to indicate how long and how much physical activity is required to burn off the corresponding energy derived from each food item. The RDA for moderately active males and females aged 20-40 years old is 2570 kcal/day and 900 kcal/day respectively. The RDA takes into account sustaining good health and providing a safety of margin in cases of illnesses and stress which depends on such factors as individual variations, food utilization and food supplies. Although it seems to take a lot of effort to work off a slice of chocolate cake containing 317 calories, what is important is the cumulative effect of regular physical activity. In other words, you need not burn off all those calories in one long physically exhausting session. A daily physical regimen will help you expend excess calories and therefore keep you in healthy caloric balance.

ENERGY EQUIVALENTS OF FOOD EXPRESSED IN MINUTES OF ACTIVITY

	FOOD ITEM	Approximate Measure	E.P.WT (gms)	Energy (kcal)	Sitting Quietly		Stretching & Flexing		Walking at own pace		Brisk Walking		Cycling		Jogging	
					M	F	M	F	M	F	M	F	M	F	M	F
A	Beverages															
	Softdrinks	1 bottle	237	95	67	86	31	37	29	38	10	26	19	23	16	20
	Beer	1 bottle	320	163	115	148	53	64	50	66	33	45	32	40	27	35
	Magnolia mango juice	1 tetra pack	250	140	99	127	46	55	43	57	28	39	28	34	23	30
	Chocolait	1 glass	240	58	112	144	52	62	49	64	32	44	31	38	26	34
B	Bread and other Bakery products															
	Pan americano	2 pc 9x8x1 cm	90	100	71	91	33	39	31	40	20	28	20	24	17	2
	Pandesal	1 pc 7x5 cm	30	66	68	87	31	38	30	39	18	27	19	23	16	20
	Cheese cake	1 piece	40	166	117	15	54	65	51	67	33	46	33	40	27	35
	Chocolate cake	1 sl 5 75x3 75x4cm	86	317	224	289	104	125	97	128	63	86	63	77	52	68
C	Sugar and sugar products															
	Sugar white	1 tbsp	12	46	33	42	5	18	14	19	9	13	9	11	8	10
	Hershey's kisses	5 pcs	20	72	51	66	24	28	22	29	14	20	14	17	2	15
	Ice cream plain	1/2 cup	80	186	1 7	151	64	65	51	67	33	46	33	40	27	35
D	Eggs, Milk & Dairy products															
	Balut	1 piece	60	109	77	99	36	43	34	44	22	30	22	26	18	23
	Chicken egg boiled	1 piece	50	88	62	80	29	35	27	36	18	24	17	21	5	9
	Evap milk undiluted	/2 cup	120	168	119	153	55	68	52	68	34	47	33	41	28	36
	Powdered milk whole	1 tbsp	8	39	28	35	3	15	12	16	8	11	8	9	6	8
	Cottage cheese	1/4 cup	45	41	29	37	13	18	13	17	8	11	8	10	1	9
	Cream, heavy whipping	1 tbsp	15	55	39	50	18	22	17	22	11	15	1	13	9	12
	Cow's milk, whole, fresh	1 glass	240	265	209	269	97	116	91	19	59	82	59	72	49	63

ENERGY EQUIVALENTS OF FOOD EXPRESSED IN MINUTES OF ACTIVITY

FOOD ITEM	Approximate Measure	E P.WT (gms)	Energy (kcal)	Sitting Quietly		Stretching & Flexing		Walking at own pace		Brisk Walking		Cycling		Jogging	
				M	F	M	F	M	F	M	F	M	F	M	F
E Fats Oil & Related Products															
Coconut grated	2 tbsp	25	78	55	71	26	31	24	32	16	22	15	19	13	7
Butter	1 tbsp	15	98	76	99	35	43	33	44	22	30	2	26	18	23
Chicharon	1 pc 6x8 cm	10	64	45	58	21	25	20	26	13	18	13	16	11	4
F Fruit & Fruit Products															
Apple red skin	1 small	75	45	32	41	15	18	14	18	9	13	9	11	7	10
Banana lakatan	1 medium	40	44	31	40	13	17	14	18	9	12	9	11	7	9
latundan	1 medium	47	48	33	39	14	17	13	17	9	12	9	9	7	9
Fruit cocktail	1/4 cup	50	52	37	47	7	21	16	21	10	14	10	13	9	1
Seedless raisins	1/4 cup	30	82	65	84	30	36	28	37	18	26	18	22	15	20
G Meat, Fish, Poultry & Products															
Porkchop, fried	1 slice	100	249	176	227	82	96	77	101	50	69	49	60	41	53
Chicken leg, fried	1 piece leg	100	237	168	216	78	93	73	96	47	66	47	58	39	5
Fishballs, deep fried	1 piece	6	16	11	15	5	6	5	6	3	4	3	4	3	3
H Other Food Items															
Chiz curls	1 pack	16	82	44	56	20	24	19	25	12	17	12	15	10	13
Jollibee regular Hamburger	1 piece	101	279	192	248	89	107	84	110	54	76	54	66	45	58
French fries	1 serving egular	75	243	72	221	80	96	75	98	49	66	48	59	40	52
Pizza pie fiesta	1/8 slice	32	86	61	78	28	34	26	35	17	24	17	21	14	18
Spaghetti w/meatballs	1 serving	85	71	50	65	23	28	22	29	14	20	14	17	12	15
Dunkin donut sugar raised	1 piece	44	199	134	172	62	75	58	76	38	53	39	46	31	40
M Chips	1 pack	45	234	56	2 3	77	92	72	95	47	65	46	57	39	50

CHROMOSOME CONSTITUTION AND ESSENTIAL OIL CHARACTERIZATION IN *COLEUS* LOUR

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ABSTRACT

Chromosome analysis conducted on Coleus laciniatus Benth. (2n = 48) and C. parviflorus Benth. (2n = 72) show that they are polyploids. Karyomorphometrical data reveal their symmetrical and primitive karyotype, which seems to be correlated with their essential oil quantification and chemical characterization. Major components found in C. laciniatus are l-limonene and [illegible]-humulene. Whereas in C. parviflorus, [illegible]-thujone and [illegible]-farnesene are the active principles.

INTRODUCTION

Coleus Lour. (Lamiaceae) is an Old World genus, commonly known as the flame nettle and possess 150 species (Morley, 1970) and are mainly distributed in the Eastern Hemisphere. This genus seems to be especially abundant in Africa, India, the Malayan Archipelagos, extending to Australia and the Pacific islands (Bailey, 1960). The genus consists of a variety of pot and garden ornamentals and plants with edible tubers. Some members are found to be of very high medicinal value (Guerrero, 1921; Burkill and Haniff, 1930; Mesdunyan et al., 1984).

Detailed karyomorphometrical studies and GC analysis of essential oil have been conducted on *C. laciniatus* Benth. and *C. parviflorus* Benth. Previous cytological studies on these plants are restricted to chromosome counts only (Mukherjee, 1959; Morton, 1962; Ramachandran, 196[illegible]). Studies on the genetic constitution and its correlation with the biosynthesis of essential oil has not been attempted so far. Therefore, the present study deals with these objectives in order to exploit the medicinal and aromatic principles found in these plants.

MATERIALS AND METHODS

Germplasm collections were made from the wild as well as cultivated areas of tropical South India; voucher specimens are deposited in the Herbarium of Sacred Heart College, Thevara, Cochin, Kerala, India.

Karyomorphological analysis:

Mitotic squash experiments were conducted on young healthy root tips pretreated with cytostatic chemicals. A solution of saturated aqueous para-dichlorobenzene with a trace of aesculine and a pinch of saponin was found to be most effective. Pretreatment was carried out at 0-5°C for 5 min. and then at 12-14°C for 2 1/2 h. They were then fixed in 1:3 Carnoy's solution overnight followed

by the aceto[illegible] or [illegible] squash technique (Sharma and Sharma 1980). In all the karyotypes [illegible] [illegible] was determined after [illegible] followed by the total centromeric index value (TF%) [illegible] were [illegible]. The [illegible] (variation coefficient) of the chromosome complement was calculated after Verma (1980).

Essential oil characterization

Essential oil [illegible] isolated from the dried [illegible] and [illegible] leaves by hydro-distillation in a Clevenger apparatus [illegible] at [illegible] analysis was performed by using a Chemito [illegible] gas chromatograph equipped with an FID and a Shimadzu Chromatopac C-R[illegible]A integrator. [illegible] used were the following: [illegible] stainless steel 2.5m x 3.0mm [illegible] 80-100 mesh [illegible] packed with [illegible]% SE-30; operating conditions: temperature programmed from [illegible] to 2[illegible] at [illegible] and isothermal at 2[illegible]; integrator and detector temperature 2[illegible]; carrier gas [illegible] inlet pressure 10 [illegible]; sample injection [illegible] ratio [illegible] sample volume 0.[illegible]. The percentage composition of oil samples were computed from the [illegible] peak areas without using correction factors. Major components were analyzed by relative retention time analysis and peak enrichment by co-injection with authentic standards.

RESULTS AND DISCUSSION

The chromosome complement was found to be a tetraploid in *C.* [illegible] (2n = 48) and a hexaploid in *C.* [illegible] [illegible] originating from the base number of X2 = [illegible] chromosomes. The secondary base chromosome number X2 = 12 is well established in the genus [illegible]. This higher basic chromosome number might have derived from its lower number X1 = 6 through [illegible] at the basic level [illegible].

The karyotypes in both taxa are characterized by the extremely small size of the chromosomes [illegible] 1.2 [illegible] decrease in [illegible] size with polyploidy, however, has been attributed [illegible] elimination of heterochromatic segments (Sharma 19[illegible]). [illegible] number [illegible] The karyotype formula shows 2 pairs of SAT chromosomes in *C.* [illegible] and [illegible] pair [illegible] followed by homogeneous small chromosomes with nearly submedian [illegible] centromeres. Thus both karyotypes appear to be symmetrical. Moreover, the lesser range of chromosome length [illegible] 1.2 [illegible] variation coefficient and high [illegible] percentage [illegible] values [illegible] their karyotype symmetry in angiosperms [illegible] of symmetrical karyotypes [illegible] (Stebbins, 1971).

The essential oil of both taxa were found to be pale [illegible] yellow in colour with the following physical characteristics: [illegible] = 1.455; *C.* [illegible] d = 0.91[illegible] [illegible] 1.4[illegible]. The major chemical constituents found in *C.* [illegible] are [illegible] and [illegible] monoterpenes and sesquiterpenes respectively. Whereas in *C.* [illegible] the major components were found to be the monoterpene [illegible] and sesquiterpene [illegible] farnesene.

by the aceto-orcein squash techniques (Sharma and Sharma, 1980). In all the karyotypes, [illegible] percentage was determined after [illegible] et al. [illegible] followed by the [illegible] asymmetry index value [illegible]. The variation coefficient of the chromosome complement was calculated after Verma (1980).

Essential oil characterization

Essential oil was isolated from the dried, flowered and [illegible] leaves by hydrodistillation in a Clevenger apparatus for [illegible] h at [illegible]. GC analysis was performed by using a [illegible] gas chromatograph equipped with an FID and a Shimadzu Chromatopac C-R [illegible] A integrator. The conditions used were the following: column – stainless steel [illegible] m x [illegible] mm [illegible], [illegible] mesh [illegible] packed with 5% SE-30; operating conditions – temperature programmed from [illegible] to [illegible] at [illegible]/min and isothermal at [illegible]; integrator and detector temperature [illegible]; carrier gas – [illegible]; inlet pressure [illegible] psi; flow rate [illegible] ml/min; sample injection – split ratio [illegible] 1:100; sample volume [illegible]. The percentage composition of oil samples were computed from the GC peak areas without using correction factors. Major components were analyzed by relative retention time analysis and peak enrichment by comparison with authentic standards.

RESULTS AND DISCUSSION

The chromosome complement was found to be a tetraploid in [illegible] (2n = 48) and a hexaploid in [illegible] (2n = 72) originating from the base number of [illegible] chromosomes. The secondary base chromosome number x_2 = 12 is well established in the genus [illegible] and [illegible]. This higher base chromosome number might have derived from its lower number x = 6 through [illegible] of the basic [illegible] genome.

The karyotype in both taxa are characterized by the extremely small size of the chromosomes (Figs. [illegible]). Decrease in chromosome size with polyploidy, however, has been attributed to either [illegible] elimination of heterochromatic segments (Sharma [illegible]) or reduction in the [illegible] number [illegible]. Karyotype formula shows [illegible] pairs of [illegible] chromosomes in [illegible] and [illegible] pairs in [illegible] [illegible] characterized by homogeneous [illegible] chromosomes with nearly submedian to nearly median centromeres. Thus both karyotypes appear to be symmetrical. Moreover, the lesser range of chromosome length [illegible], the variation coefficient and [illegible] total forma percentage [illegible] (Table [illegible]) values confirm their karyotype symmetry. In angiosperms, [illegible] symmetrical karyotypes [illegible] primitiveness (Stebbins, 1971).

The essential oil of both plants were found to be pale [illegible] yellow in color with the following physical [illegible]: [illegible] = 1.[illegible]; [illegible] [illegible]. The major chemical constituents found in [illegible] are [illegible] and [illegible], and [illegible] respectively. Whereas in [illegible] the major constituents were found to be the [illegible] and [illegible].

Thus both plants belong to mixed chemotypes. The comparatively low percentage of prime constituents (vide Table 1) show the relative complexity of their essential oils. The quality of the oil and the quantitative composition of major constituents are genetically controlled (Heffendeh and Murray 1978) and attributed to their respective biosynthetic pathways (Erdtman 1962). The terpenoids present in these plants could well be exploited through breeding techniques which may improve the qualitative as well as quantitative characteristics of their essential oil.

SUMMARY

The karyotype of *C. lacinialus* and *C. parviflorus* exhibit polyploidy with 2n=48 (4x) and 2n = 72 (6x) respectively. The various karyomorphometrical data show their relative primitive wild characteristics. Moreover both plants belong to mixed chemotypes showing the presence of various mono- and sesquiterpenoids. The predominant vegetative means of propagation found in these plants together with higher levels of ploidy and a primitive genetic setup could be exploited by conducting breeding experiments. Thus new breeds of high yielding plants can be developed as a commercial source of terpenoids of medicinal and economic importance.

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Table 1. Karyotype and essential oil data on two species of *Coleus* Lour.

Name of the taxa	2n	PL	KF	VC	TF%	Oil%	Major Components
C. forskolei Benth.	48	4x	A4B44	13.41	41.51	0.05	β - Ionone (16%), α - Humulene (9.2%)
C. parviflorus Benth.	72	6x	A6B66	12.83	41.9	0.04	β Thujone (9%), α - Farnesene (8.1%)

2n = Somatic chromosome number
PL = Ploidy level
KF = Karyotype formula.
VC = Variation coefficient
TF% = Total forms percentage.

Figure 1. Karyotype of *Coleus lacinialus*. Scale represents 10 µm.

Figure 2. Karyotype of *Coleus parviflorus*. Scale represents 10 µm.

EGG PARASITOIDS OF COTTON BOLLWORM, *HELICOVERPA ARMIGERA* (HUBNER) IN SELECTED COTTON GROWING AREAS

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ABSTRACT

The study was conducted in selected cotton growing areas of Batac and Bacarra, Ilocos Norte and at the Cotton Research and Development Institute Experimental Farm in San Juan, Ilocos Sur from November 1991 to May 1993. It aimed to survey the naturally occurring cotton bollworm egg parasitoids, to determine their degree of parasitization in the field and to compare the bollworm egg parasitization between sprayed and unsprayed plots.

The collected naturally occurring egg parasitoid in Batac and Bacarra was identified as Trichogramma chilonis *(Ishii), where parasitization ranged from [illegible] to [illegible]% with an average of 47.87%.*

The egg parasitoid thrived throughout the year where it was also present in the preceding rice crop attacking stem borer and army worm eggs.

In San Juan, Ilocos Sur, two egg parasitoids were identified: Trichogramma chilonis *(Ishii) and* Trichogrammatoidea nana *(Nagaraja). The degree of parasitization of bollworm eggs did not vary greatly in sprayed and unsprayed plots. However, their incidence differed at various developmental stages of the cotton crops whereby there was a low parasitization of bollworm eggs noted in the early squaring stage. In contrast, high parasitization was observed at later stages.*

INTRODUCTION

The adverse effects of synthetic insecticides justified the renewed efforts on the use of biological control agents such as egg parasitoids have the advantage over other biological control agents because the target pest is killed before it could cause damage to the crop.

In the Philippines, egg parasitoids can effectively control lepidopterous pests. Its use started in 1934 with imported *Trichogramma* species (Baltazar 1963). The potentials of indigenous species were ignored until Alba and Estioko (1980) and Nagaraja (1984) reported that indigenous species were also effective against sugar cane and pod borers, respectively in succeeding studies. Alba (1988) reported that four *Trichogramma* and six *Trichogrammatoidea* were attacking lepidopterous eggs in different host plants. Despite the importance of biological control, there is no serious effort made so far in studying the naturally occurring egg parasitoids and their potentials as control agents for cotton pests. Hence, this study was conducted 1) to survey the naturally occurring egg parasitoids of cotton bollworm in selected cotton

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growing areas 2) to determine their degree of parasitization in the field, and 3) to compare the bollworm egg parasitization between sprayed and unsprayed areas.

MATERIALS AND METHODS

Survey of Egg Parasitoids

Ten cotton fields in Batac and Bacarra, Ilocos Norte were surveyed and about 80 to 100 bollworm eggs were collected from each site. The eggs were collected by tearing the portion of the leaf where these were attached. Then were placed individually in glass vials plugged with cotton balls. The collected eggs were brought to the laboratory for further observations. Those that hatched were discarded. However, blackened eggs which did not hatch were further observed for 13 days after collection. Emerging parasitoids were preserved in 70% alcohol. The collected parasitoids were identified through the help of Dr. Jean Voegele, a consultant of the Philippine German Biogica Crop Protection Project. Data on sampling location, number of parasitized eggs and the age of the crop were also taken.

A survey was also made to determine the occurrence of egg parasitoids during off season planting of cotton. It was conducted in the preceding rice crop in Nalo, Rana West and PAPI fields. Eggs of stem borer and army worm were collected. The procedure used in the collection of bollworm eggs was also followed.

Comparison of Sprayed and Unsprayed Plots

An area of 2 000 square meters located at the Cotton Research and Development Institutes' San Juan Experimental Station was used for the conduct of this study. The plot was divided into two, one that was sprayed with chemical insecticides and the other was unsprayed. The plants were sprayed at 32, 43, 56, 66, 75, 82 and 92 days after emergence (DAE) and was based on the Critical Pest Level. Eggs were collected at 35, 50, 65 and 90 DAE. The procedure in the preceding study was also employed. The data obtained were similar as that of study I. Seedcotton yield and yield components were gathered at harvest.

RESULT AND DISCUSSION

Survey of Egg Parasitoids

The parasitoids emerged from the blackened bollworm eggs after seven to thirteen days after collection (Table 1). This indicates that oviposition by the parasitoids took place in different days. The number of parasites emerged per egg ranged from one to seven and 56.42 % were females while 43.58 % were males.

The collected egg parasites from cotton growing areas in Batac and Bacarra, Ilocos Norte was identified as *Trichogramma chilonis* Ishii while those from San Juan, Ilocos Sur were *T. chilonis* (Ishii) and *Tetrastichogrammatoidea conjugata* (Nagaraja).

The presence of naturally occuring parasitoids could be influenced by the cropping pattern and crop diversity. In Ilocos Norte the preceeding crop was rice which is the source of the parasitoids. Rice stemborer and army worm in rice plants were also the hosts of *Trichogramma* (Table 2). This finding implies that the egg parasitoid thrives throughout the year. The time gap between harvesting of rice and the planting of cotton is about two to three weeks while the interval between the harvesting of cotton to the planting of rice is about four to six weeks. Vongsis (1993) pointed out that Trichogramma could survive at about four weeks under normal tropical condition. Furthermore, some areas near the sampling sites were planted earlier with tomato and corn instead of cotton after the rice crop was harvested. Since tomato and corn crops are hosts of the cotton bollworm, the parasitoids persist even without the cotton plant as plant host.

In San Juan, Ilocos Sur, rice and corn were planted earlier near the experimental area and *Trichogramma* could have possibly migrated to said study site.

Degree of parasitization

Parasitized eggs ranged from 18.18% to 82.76% with an average of 47.97 ± 22.04 (Table 3). This indicates that the naturally occurring *Trichogramma chilonis* in cotton areas in Batac and Bacarra, Ilocos Norte had high population density despite frequent spraying (about six to 10 times) of insecticides by the farmers. Furthermore, this could be attributed to the diversity of crops in these areas. There was migration of egg parasitoids from one crop to another especially from unsprayed crops or crops planted earlier where the parasitoids have already established their population.

The percentage of eggs that hatched as larvae was quite low (28.94%). However, 23.08% of the eggs did not hatch and could be due to some factors such as egg sterility, effect of insecticide, egg predation and other natural factors.

Comparison of sprayed and unsprayed plots

The degree of parasitization did not greatly vary between sprayed and unsprayed plots (Fig. 1). Parasitoids from unsprayed plot probably transferred to the sprayed plot, hence contributing to an increase egg parasitization. Furthermore, rice and corn were grown near the experimental site and parasitoids could have possibly transferred to said study site.

However, the seed cotton yield harvested from the sprayed plot was higher than the yield on unsprayed plot by 14.23% (Table 4). The difference on yields between treatments was attributed to lesser number of developed early bolls on unsprayed plants. This could be due to low bollworm egg parasitization at early stage of the crop that contributed to high population of bollworm.

Parasitization at different plant growth stages

There was a low parasitization of bollworm eggs at early squaring stage of the crop (35 DAE). This could be due to the low initial population of the naturally-occuring parasitoids in the field. However, as the crop matured, the population increased tremendously as exhibited by high bollworm egg parasitization during peak squaring, flowering and boiling stages.

With this result there is a need to supplement the naturally-occurring parasitoids during the early stage of the crop. However as the population of the parasitoids become established at later stage, there is no more need to supplement the existing Trichogramma in the field.

SUMMARY AND CONCLUSION

The study was conducted in cotton growing areas in Batac and Bacarra, Ilocos Norte and at CRDI Experimental Station, San Juan, Ilocos Sur from November 1991 to May 1993. The study surveyed the naturally occurring egg parasitoids of cotton bollworm, determined their degree of parasitization in the field and compared the bollworm egg parasitization between sprayed and unsprayed plots.

The naturally occurring *Trichogramma chilonis* Ishii was observed to attack bollworm eggs in Batac and Bacarra, Ilocos Norte. The parasitization ranged from 18.18 to 82.76% with an average of 49.97% in the farmers' field despite frequent spraying with chemical insecticide.

In San Juan, Ilocos Sur, two bollworm egg parasitoids were identified namely *Trichogramma chilonis* Ishii and *Trichogrammatoidea cojuangcoi* (Nagaraja). The degree of parasitization of bollworm eggs did not vary greatly in sprayed and unsprayed plots. However, it is recommended that some areas must be left unsprayed so that there would be sources of inoculum of the parasitoids.

Low parasitization was observed at the early squaring of the cotton crop. However, high parasitization was observed at later stages of the crop. This implies that the initial population of the parasitoid in the field was very low but as the crop matured, the population increased. This result indicates that inoculation of bollworm egg parasitoid is necessary during the early stage of the crop.

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Table 1. Number of days from collection to blackening and *Trichogramma* emergence, number of *Trichogramma* per eggs and percentage of female and male.

PARAMETERS	RANGE	MEAN
Number of days from collection to blackening	2 - 3	2.52
Number of days from collection to *Trichogramma* emergence	7 13	9.63
Number of *Trichogramma* per egg	1 - 7	2.69
Percentage of female *Trichogramma*	56.42	
Percentage of male *Trichogramma*	43.58	
Number of bollworm eggs sampled	110.00	

Table 2. Degree of lepidopterous egg parasitization of the naturally occuring *Trichogramma* chilonis in rice crop in three Batac cotton growing areas.

Crop Stage	Place of Collection	No. of Eggs Collected	Percent Parasitization
Early Tillering Stage	Baca West	4	50.00
	Nolo	3	66.00
	CRDI, CES	2	[illegible]00
Late Tillering Stage	Baca West	10	50.00
	Nolo	7	71.00
	CRDI, CES	5	60.00
Flowering Stage	Baca West	12	66.00
	Nolo	8	62.50
	CRDI, CES	8	37.50
Ripening Stage	Baca West	3	33.33
	Nolo	2	50.00
	CRDI, CES	5	40.00

Table 3. Degree of bollworm egg parasitization of the naturally occurring *Trichogramma chilonis* on cotton in Batac and Bacarra cotton growing areas.

PLACE OF COLLECTION	Percent Parasitization	Percent Emerged as Larvae	Percent Non-Emergence	Crop Stage
BACARRA, ILOCOS NORTE				
Brgy #10	29.92	25.92	46.15	flowering
Caberoan	18.18	45.45	36.36	flowering
Cadanglaan 1	61.11	18.06	20.83	flowering
Cadanglaan (2)	76.40	6.74	16.85	flowering
Corbor	45.96	18.92	35.14	flowering
BATAC, ILOCOS NORTE				
Baoa West	54.54	31.82	13.64	flowering
Dariwdiw	50.00	12.50	37.50	flowering
Noto	82.76	3.45	13.97	flowering
CRDI, CES (1)	43.82	56.18	0.0	boiling
CRDI CES (2)	20.00	69.38	10.76	boiling

Table 4. Seedcotton yield and yield components of sprayed and unsprayed plots in San Juan, Ilocos Sur.

Treatment	Plant Population (ha)	Bolls/ Plant	Weight/ Boll (g)	Seedcotton Yield (kg/ha)	% Yield Reduction
Sprayed plot	88,000	4.476	4.63	1876.66	—
Unsprayed plot	87,500	4.415	4.05	1556.66	14.29

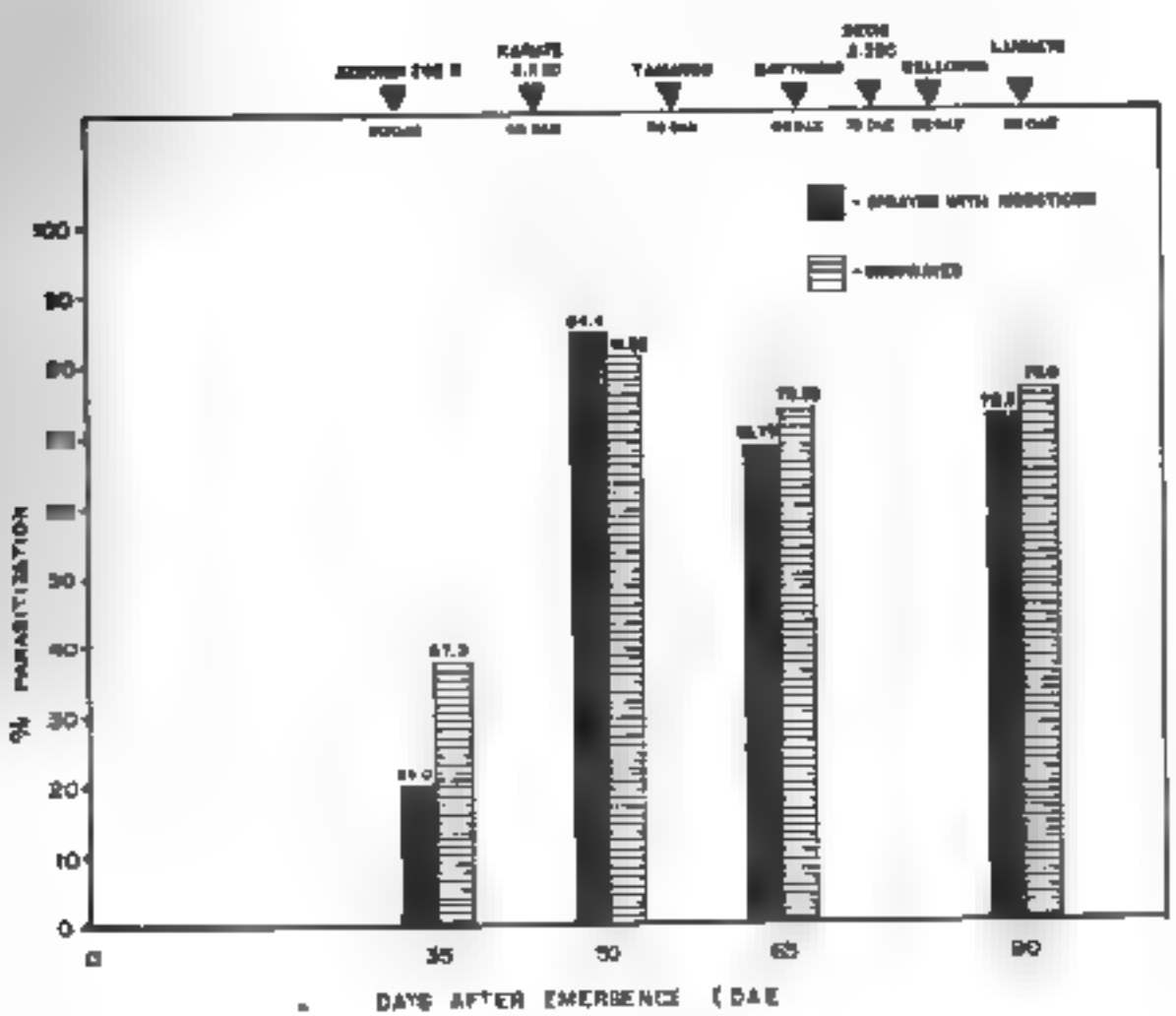

Figure 1. Degree of cotton bollworm egg paratization during the different stages of the cotton crops in sprayed and unsprayed plots.

ANTI-TUMOR PROMOTING ACTIVITY OF DECOCTIONS AND EXPRESSED JUICES FROM PHILIPPINE MEDICINAL PLANTS

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ABSTRACT

A modified tumor promotion test was used in this study. When dimethylbenzanthracene was used as the initiator and croton oil as the promoter, 67% of the experimental mice developed skin tumors, 50% had liver tumors, and 33% had colon tumors. These were observed within a period of 30 weeks.

Complete inhibition of formation of all types of tumors was exhibited by decoction from mango bark and expressed juices from leaves of guava, sulasi, kalatsutsi, yerba buena, pandan, kinchay, sabila, damong Maria, mayana and flowers of santan puti and santan dilaw.

Partial inhibition of tumor formation was exhibited by expressed juice from garlic bulbs, leaves of alagaw, flowers from gumamela and decoction from bagon roots and leaves of romero. Expressed juice from onion bulbs inhibited the development of liver tumors but not skin tumors.

It is possible that the test medicinal plants contain constituents that can inhibit the promotion stage of carcinogenesis.

INTRODUCTION

The multistage model of carcinogenesis has been proposed recently (Sugimura and Wakabayasi, 1991)

INITIATION → PROMOTION → CONVERSION

→ PROGRESSION

At the initiation stage the carcinogen reacts with DNA the genetic substance of the living cell. The reactivity may result in alteration of the structure of DNA as a consequence of reactions with amines and pyrimidine bases, intercalation with base-pairs, single and double strand breaks and deletions.

If the altered structure escapes repair, mutations result which may activate protooncogenes and inactivate tumor suppressor genes. At the promotion stage there is clonal expansion of initiated cells. At the conversion and progression stages, specific chromosomal changes are involved.

The medicinal plants selected in this study are those that have been shown to inhibit the initiation stage of carcinogenesis. It would be of great interest if it can be shown if they inhibit the second stage, the promotion stage of carcinogenesis.

MATERIALS AND METHODS

Dimethylbenzanthracene and croton oil were obtain from Sigma Chemica Company, St Louis, Missouri, U.S.A.

Swiss Webster Mice were furnished by the College of Veterinary Medicine University of the Philippines, Diliman

A modification of the skin tumor promotion test by Berenblum and Shubik, 1978 was used. The experimental mice were shaved at the back, three days before the application of the initiator dimethylbenzanthracene. Three days after the promoter croton oil, was applied on the shaved area and thirty minutes after the decoction or expressed juices of the medicinal plants were brushed on the same shaven area. This application of the promoter and the plant extracts were repeated three times a week for 20 weeks. The appearance of skin tumors was noted within this period. At the end of 20 weeks the animals were dissected and examined for the presence of organ tumors.

RESULTS AND DISCUSSION

Table 1 indicates the scientific names and the parts of plants which decoction were used in the study.

Table 2 shows scientific names and parts of the plants which expressed juices were used as test systems.

The tumor promoting activity of croton oil with dimethylbenzanthracene as the initiator is shown in Table 3.

Croton oil alone or dimethylbenzanthracene alone (DMBA) did not induce the formation of tumors. Although DMBA a carcinogen, alkylates DNA after metabolic activation a promoter is needed to induce clonal expansion of initiated cells. Croton oil alone without the initiator will not have initiated cells which clonal expansion it can induce.

The antitumor promoting activity of decoction from five plants are shown in Table 4. Complete inhibition of skin and organ tumors was shown by decoction from leaves of Isaang gubat and bark from mango. Decoction from seeds of ipil-ipil inhibited completely the formation of skin tumors and reduced the development of organ tumors to a significant extent. Decoction from roots of kogon and leaves of romero inhibited completely the development of organ tumors and reduced to a significant extent the formation of skin tumors.

Table 5 shows the antitumor promoting activity of expressed juices from fifteen medicinal plants. Expressed juices from leaves of guava, suasi, kakaiauta, yerba buena, pandan, kinshay, sabila, damong maria, and mayana inhibited completely the formation of skin and organ tumors. This was also shown by the expressed juices from red and yellow flowers from aantan. Partial but significant reduction of formation of skin tumors was shown by expressed juice from garlic bulbs, from

alagaw leaves and from flowers of gumamela. Expressed juice from onion bulbs reduced the extent of liver tumor formation but did not inhibit skin tumor development.

These results suggest that for most of the plants used, they possess constituents which can inhibit the second stage which is the promotion stage of carcinogenesis.

CONCLUSION

Of the plants studied only expressed juice from onion bulbs did not inhibit skin tumor formation. All others showed either complete inhibition or partial inhibition of skin tumor formation. Except for decoction from ipil-ipil and expressed juice from onion bulbs, all other preparations from the test plants showed complete inhibition of organ tumors. Only partial inhibition was shown by decoction from ipil-ipil seeds and expressed juice from onion bulbs.

ACKNOWLEDGEMENT

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Table 1. Scientific names of plants which decoctions were used.

Local Name	Scientific Names	Parts Used
Ipil-ipil	*Mimosa glauca* Linn.	seeds
Kogon	*Imperata koenigii* Beauv.	roots
Mango	*Mangifera indica* Linn.	bark
Romero	*Rosmarinus officinalis* Linn.	leaves
Tsaang gubat	*Ehretia mollis* Merr.	leaves

15% decoctions were made of each

Table 2. Scientific names of plants which expressed juices were used.

Local Name	Scientific Names	Parts Used
Alagaw	*Premna nauseosa* Blanco	leaves
Damong Maria	*Artemesia vulgaris* Linn.	leaves
Garlic	*Allium sativum* Linn.	bulb
Guava	*Psidium guajava* Linn.	leaves
Gumamela	*Hibiscus rosasinensis* Linn.	flowers
Kalatsutsi	*Plumiera acuminata* Air.	leaves
Kinchey	*Apium graveolens* Linn.	leaves
Mayana	*Coleus blumei* Benth.	leaves
Onion	*Allium cepa* Linn.	bulb
Pandan	*Pandanus odoratissimus* Linn.	leaves
Sabila	*Aloe vera* Linn.	leaves
Santan dilaw	*Ixora chinensis* Linn.	flowers
Santan pula	*Ixora coccinea* Linn.	flowers
Sulasi	*Ocimium sanctum* Linn.	leaves
Yerba buena	*Mentha cordifolia* Linn.	leaves

Table 3. Tumor Promoting Activity of Croton Oil with Dimethylbenzanthracene.

	% Skin Tumors	% Organ Tumors
Croton Oil + DMBA*	67%	50 % (liver) 30 % (colon) 16 % (oral)
Croton oil alone	0	0
DMBA alone	0	0

* dimethylbenzanthracene

Table 4. Antitumor Promoting Activity of Decoctions.

	% Skin Tumors	% Organ Tumors
DMBA + croton oil	67%	50% (liver)
plus ipil-ipil	0	14%
plus kogon	14%	0
plus mango	0	0
plus romero	14%	0
plus tsaang gubat	0	0

Table 6. Antitumor Promoting Activity of Expressed Juices.

	% Skin Tumors	% Organ Tumors
DMBA + croton oil	67%	50% (liver)
plus alagaw	12%	0
plus damong Maria	0	0
plus garlic	33%	0
plus guava	0	0
plus gumamela	12%	0
plus kalatsutsi	0	0
plus kinchay	0	0
plus mayana	0	0
plus onion	67%	33%
plus pandan	0	0
plus sabila	0	0
plus santan dilaw	0	0
plus santan puta	0	0
plus sulasi	0	0
plus yerba buena	0	0